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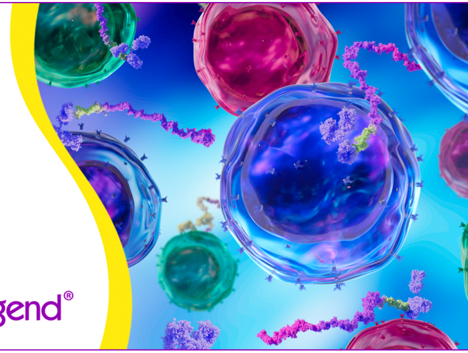

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METABOLIC HETEROGENEITY AMONG HUMAN MONOCYTES AND ITS MODULATION BY PGE_2

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Human peripheral blood monocytes (Mϕ) were fractionated on a discontinuous bovine serum albumin density gradient, and cells from each fraction was assayed for their specific activity of two enzymes, which represent correlates of Mϕ activation or differentiation (5'-nucleotidase, acid phosphatase) and their synthesis of prostaglandin E_2 (PGE_2). On the basis of significant differences in these parameters, the monocytes could be divided into two broad populations. Low density cells demonstrated low specific activities of 5'-nucleotidase (5'N), high specific activities of acid phosphatase (APT'ase), and synthesized substantial amounts of PGE_2. In contrast, high density cells demonstrated high specific activities of 5'-N, low specific activities of APT'ase, and synthesized meager amounts of PGE_2.

To determine if these biochemical differences served as stable markers of Mϕ subpopulations, low and high density Mϕ were individually cultured for 3 days, and changes in the metabolic parameters were assayed. Although 5'-N and APT'ase increased among each population, the magnitude of this change could be modulated by PGE_2. When each population was cultured in a concentration of PGE_2 equivalent to that synthesized by the whole Mϕ, differences in 5'-N and APT'ase remained as useful discriminators of the Mϕ subpopulations. The pertinence of these findings to events involved in Mϕ activation and differentiation as well as a possible role for PGE_2 in modulating these events is discussed.

Cells of the monocyte/macrophage line (Mϕ)[3] are required for the initiation and expression of immunity. This requirement includes such diverse capabilities as suitable presentation of activating determinants (1, 2), regulation of immunocyte activation and differentiation (3, 4), and serving as an intermediary through which other regulatory cells may exert their effect (5, 6). It is possible that all Mϕ are equally capable of mediating these reactivities. Alternatively, these functions may be distributed among distinct subpopulations within the Mϕ line. This latter possibility is suggested by studies in animal models demonstrating populations of Mϕ that can be distinguished by differences in their cell surface markers (7–9), metabolic activity (10), liberation of immunoregulatory materials (11), and determinant presentation (12).

The recent development of techniques useful for the isolation and cultivation of human peripheral blood Mϕ has enabled similar studies to determine the existence of distinct populations within these Mϕ (13). We have recently reported that not all peripheral blood Mϕ are equivalent in their ability to serve as accessory cells for the concanavalin A-induced generation of suppressive T cells (14). In this manuscript, we demonstrate that Mϕ also differ in certain metabolic parameters. Based on significant differences in their specific activities of 5'-nucleotidase (5'-N) and acid phosphatase (APT'ase, two enzymes that represent correlates of Mϕ activation and differentiation; 15–17) and their synthesis of prostaglandin E_2 (PGE_2), Mϕ were broadly divided into two populations. One population demonstrated a low specific activity of 5'-N, a high specific activity of APT'ase, and substantial synthesis of PGE_2. The other manifested a relatively high specific activity of 5'-N, a low specific activity of APT'ase, and meager synthesis of PGE_2. Short-term culture was accompanied by changes in the specific activity of each enzyme among both populations, and these changes were modulated by PGE_2. If each population was cultured in equivalent concentrations of PGE_2, differences in 5'-N and APT'ase remained as markers for distinguishing the Mϕ populations.

MATERIALS AND METHODS

Isolation of adherent Mϕ. Populations enriched for Mϕ (>95% esterase positive, >90% phagocytic) were obtained by adherence as follows. Peripheral blood mononuclear cells (PBMC; 72 ± 4% T, 6 ± 2% immunoglobulin bearing, and 18 ± 8% esterase positive; 18) were separated from platelets (a major source of prostaglandins) by first washing the cells twice in CA^{++}-, MG^{++}-free buffer containing 0.5 mM EDTA[3] and then sedimenting them through 5% bovine serum albumin (BSA; Miles Labs, Kankakee, Ill.; platelets remained at the top of the BSA, whereas mononuclear cells sedimented to the bottom). This yielded a monocyte population that, by microscopic examination, contained less than one platelet per 10 PBMC. The recovered cells were suspended in RPMI 1640 (Cell Culture Facility, U.C.S.F.) containing nonheat-inactivated autologous serum (10%), and 3×10^6 cells were placed in 16-mm culture wells (Costar, Cambridge, Mass.) containing a prewashed, glass coverslip. After a 1-hour incubation at 37°C, nonadherent cells were removed by vigorous washing.

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[3] Abbreviations used in this paper: Mϕ, monocytes/macrophages; 5'-N, 5'-nucleotidase; APT'ase, acid phosphatase; PGE_2, prostaglandin E_2; HAS, heat-inactivated autologous serum; PBMC, peripheral blood mononuclear cells; EDTA, ethylenediaminetetraacetic acid. NP-40, Nonidet P-40; RIA, radioimmunoassay.

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Gradient fractionation of monocytes was performed as previously described (14). Coverslips containing adherent PBMC were incubated for 10 min at 23°C in 10% autologous serum containing lidocaine (24 mM final concentration, Astra Pharmaceuticals, Farmington, Mass.). The rounded cells were detached by vigorous washing (this represented 12% of the original PBMC and 90% of the cells that had initially adhered to the coverslip) and sedimented through a five-step discontinuous gradient consisting of 17%/19%/21%/23%/25% BSA (Pathocyte 5, Miles Labs). The recovered cells (82 ± 5% of those originally placed on the gradient) were divided into the following four fractions: fraction 1 = 17%/19% interface, fraction 2 = 19%/21% interface, fraction 3 = 21%/23% interface, fraction 4 + 5 = 23%/25% interface and pellet, with 19.3 ± 4.7%, 18.6 ± 4.5%, 22.8 ± 3.9%, and 39.7 ± 2.2% (mean ± S.E. for 10 gradients) of the recovered cells obtained in the four fractions, respectively. All fractions contained greater than 95% viable monocytes as determined by trypan blue exclusion, esterase staining, ingestion of latex particles, and transmission electron microscopy (kindly performed by Dr. Dorothy Bainton).

Short-term culture (3 days) of Mϕ was performed at 37°C in a humidified atmosphere of 95% air, 5% CO_2 with RPMI 1640 containing 10% heat-inactivated autologous serum (HAS; 56°C for 30 min) used as the culture medium.

Enzyme assays. Total cellular protein as well as specific activities of 5′-N and APT′ase were performed on several different populations, including 1) PBMC that had adhered to glass coverslips, 2) fractionated cells removed directly from the gradient, and 3) fractionated cells that were readhered to glass coverslips. For adherent populations, the coverslips were placed directly into glass vials containing 0.15 ml of 0.1% Nonidet P-40 (NP-40; Particle Data Labs, Elmhurst, Ill.). Cells in suspension were first pelleted by centrifugation, and NP-40 was added directly to the cell pellet. In both cases, the cells were lysed by five cycles of freeze-thawing.

Protein determinations were performed on the cell lysate utilizing the method of Lowry *et al.* (19). BSA served as the protein standard.

5′-N activity was determined by measuring the conversion of ^{3}H-AMP to soluble ^{3}H-adenosine as described by Van der Zeijst *et al.* (20). Twenty-five, 50, or 100 μl of the cell lysate were added to varying amounts of a reaction mixture such that final concentrations were 54 mM Tris-HCl (pH 9.0), 12 mM $MgCl_2$, 0.1% NP-40, 3.8×10^{-5} M unlabeled AMP (Sigma, St. Louis, Mo.), and 2.25×10^{-9} M (0.056 μCi) labeled AMP (adenosine-2-^{3}H-5-monophosphate, ammonium salt, 18.5 Ci/mM, Amersham Searle, Arlington Heights, Ill.) in a final volume of 1.0 ml. 8×10^{-5} M β-glycerophosphate, a competitive inhibitor of nonspecific phosphatase, was routinely included in the assay solution to insure that the reactivity measured was 5′-N. After a 2-hr incubation at 37°C, the reaction was terminated by the addition of 0.15 ml each of 0.25 M $ZnSO_4$ and 0.25 M $Ba(OH)_2$, and the unreacted, precipitated ^{3}H-AMP was sedimented by centrifugation. The amount of reaction product generated was determined by dividing the counts per minute in 0.5 ml of the supernatant by the total counts per minute present in the reaction mixture. The generation of ^{3}H-adenosine was linear, utilizing the three different volumes of the monocyte lysate. Results are expressed as specific activity, i.e., μg of product generated/hr/mg of cell protein.

Monocyte APT′ase was determined by measuring the conversion of α-napthyl acid phosphate to α-napthol as described by Allen and Gockerman (21). Twenty-five, 50, or 100 μl of the cell lysate were mixed to a final volume of 1.0 ml with substrate (α-napthyl acid phosphate, 5 mM in 50 mM acetate/acetic acid buffer) for 2 hr at 37°C. The reaction was terminated by the addition of 0.2% diazotized Fast Red ITR (Sigma) in 10 mM barbital-HCl buffer (pH 8.0, containing 4% sodium lauryl sulfate and 100 mM sodium acetate). The Fast Red ITR/α-napthol complex was quantitated spectrophotometrically at 454 nm by using measured amounts of α-napthol as standards. The amount of substrate generated was proportional to the amount of cell lysate added. Results are expressed as specific activity, i.e., μg of product generated/hr/mg protein.

Assay of PGE_2 synthesis. To assay for monocyte synthesis of PGE_2, adherent PBMC, fractionated cells, or fractionated cells that were readhered to glass coverslips were cultured for 48 hr in 10% HAS. The culture fluid was then removed and assayed by radioimmunoassay (RIA) as previously described (22). The antibody, generated against PGE_2, demonstrated the following cross-reactivities at 50% displacement of {^{3}H} PGE_2: PGE_2 = 100%, PGE_1 = 15%, $PGE_{2\alpha}$ = 8%, PGA_2 = 0.05%, PGB_2 = 0.6%, PGD_2 = 0.05%, 6-keto-$PGF_{1\alpha}$ = <3%, thromboxane B_1 = <0.1%, and arachidonic acid = 0.003%. Platelet contribution to PGE_2 synthesis was <1 Fg/10^6 cells based on previous quantitation of PGE_2 synthesized by platelets and our observation that the cell populations assayed contained 1% platelets (23). Initial experiments demonstrated no difference in PGE_2 synthesis among fractionated cells assayed before or after a secondary adherence to glass. Thus, the PGE_2 values reported represent those obtained utilizing cells taken directly from the gradient and are presented as ng of PGE_2 equivalents/10^6 cells.

To block endogenous prostaglandin synthesis, indomethacin (Sigma) was dissolved in 7% $NaCO_3$ and added in a final concentration of 10^{-6} M to the culture media. This consistently resulted in the synthesis of less than 5 ng of PGE_2 equivalents/10^6 monocytes. Exogenous PGE_2 (a generous gift of Dr. J. Pike, Upjohn Co., Kalamazoo, Michigan; 10 mg/ml stock solution in 100% ethanol) was added to monocyte cultures in a final concentration of 10^{-7} M. This concentration was equivalent to that actually synthesized by Mϕ as determined by quantitative, gas liquid chromatographic/mass spectrometric analysis of Mϕ culture fluids (23). Controls consisted of cultures that received a comparable concentration of ETOH (<0.001%) without PGE_2.

RESULTS

Metabolic activity of fractionated Mϕ. Adherent Mϕ were fractionated on a five-step, BSA density gradient, and cells from each interface were assayed directly for a) their specific activities of 5′-N and APT′ase, and b) their synthesis on immunoreactive PGE_2 (Fig. 1). Cells sedimenting in fractions 1, 2, or 3 could not be distinguished by differences in their specific activities of either 5′-N or APT′ase. However, the activity of each enzyme among these low density cells was significantly different from that measured among high density monocytes (fractions 4 + 5; $p < 0.005$ for both 5′-N and APT′ase as determined by the one-tailed Student's *t*-test). 5′-N levels among low density cells were less than those detected among high density populations. Conversely, specific activities of APT′ase were greater among the low than among high density monocytes. Similar data was obtained when the results were calculated as specific activity/10^6 cells, indicating that the noted differences were not simply due to variations in the amount of cellular protein associated with each fraction (data not shown). In experiments not presented, cells from each gradient fraction were first readhered to glass coverslips and subsequently analyzed for specific activities of 5′-N and APT′ase. After this

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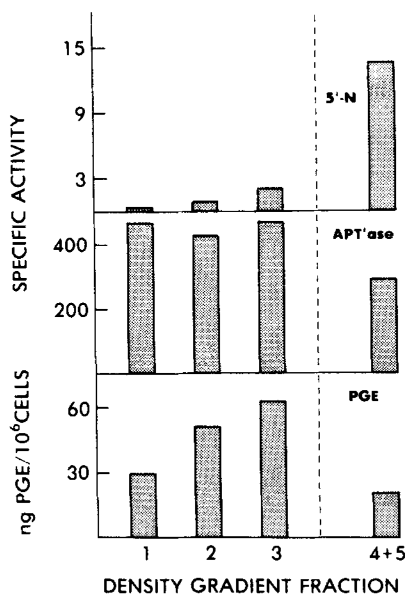

Figure 1. Heterogeneity among fractionated monocytes. Peripheral blood monocytes were fractionated on a five-step discontinuous BSA gradient and cells from each fraction were assayed for: *upper*, specific activities of 5′-N; *middle*, specific activities of APT′ase; and *lower*, synthesis of immunoreactive PGE_2. The results are representative of those obtained in three experiments.

secondary adherence, cells from each fraction contained >98% viable Mϕ, and the relative differences in their specific activities of 5′-N and APT′ase as well as PGE_2 synthesis were comparable to those presented in Figure 1. Thus, the noted profiles of 5′-N and APT′ase reflect those of fractionated Mϕ and not any contribution by other cells that might exist in the gradient fractions.

Fractionated Mϕ also differed in their ability to synthesize immunoreactive PGE_2 (Fig. 1). Again, the differences were significant ($p < 0.02$) only when the amount of PGE_2 synthesized/10^6 low density cells (fraction 1 + 2 + 3; 49.4 ± 4.8 ng) was compared with that synthesized/10^6 high density monocytes (fraction 4 + 5; 7.3 ± 5.6 ng, mean ± S.E. for five experiments). The differential synthesis of PGE_2 by low and high density Mϕ was confirmed by studies in which ^{14}C arachidonic acid (the precursor for PGE_2) was added to the Mϕ for 1 hr and the radiolabeled products were separated by thin-layer chromatography and detected by radioactivity scanning (24). This indicates that the differential PGE_2 synthesis by the Mϕ fractions was not related to differences in their survival during the 48-hr culture. Furthermore, it suggests variability in the activity of Mϕ enzymes required for PGE_2 synthesis rather than in the availability of substrate.

Thus, on the basis of significant differences in the parameters measured, fractionated monocytes could be broadly divided into two populations. Low density monocytes (fraction 1 + 2 + 3, 60% of the recovered population) manifested a relatively low specific activity of 5′-N, a high specific activity of APT′ase, and synthesized substantial amounts of PGE_2. In contrast, high density cells (fraction 4 + 5) demonstrated a high specific activity of 5′-N, a low specific activity of APT′ase, and synthesized meager amounts of PGE_2. Although absolute values for 5′-N, APT′ase, and PGE_2 synthesis among fractionated monocytes from different individuals varied, the relative differences in these parameters among low and high density populations were remarkably constant.

Culture-dependent changes in Mϕ 5′-N and APT′ase. An important question is whether the differences in 5′-N, APT′ase, and PGE_2 synthesis noted among fresh Mϕ are stable. To determine this, we utilized the finding that short-term culture of Mϕ results in an increase in their specific activities of 5′-N and APT′ase (Table I; 13, 25). Unfractionated Mϕ recovered after a 3-day incubation in 10% HAS (50% of the cells initially cultured) demonstrated a mean 40-fold increase in 5′-N and a 1.7-fold increase in APT′ase. Short-term culture of the individual low and high density Mϕ also resulted in an increase in their enzyme-specific activities. However, the relative increase in each enzyme among the two populations was not similar (recovery of viable Mϕ from the cultured low and high density populations was equivalent, 56 ± 4% and 44 ± 8%, respectively). 5′-N levels increased 38-fold among the low and only 8.1-fold among high density cells. Conversely, specific activities of APT′ase increased 1.2-fold among low and 1.8-fold among the high density monocytes. As a result of these dissimilar changes, the cultured subpopulations could no longer be distinguished by differences in their enzyme-specific activities. Although the specific activities of 5′-N among fresh low and high density cells were significantly different, those noted among the cultured populations were not (192 ± 72 *vs* 154 ± 65, $p > 0.2$). A similar situation was noted for specific activities of APT′ase (416 ± 42 *vs* 417, $p > 0.2$). Comparable relative increases in 5′-N and APT′ase occurring during a 3-day culture were noted among nonfractionated Mϕ and Mϕ that were initially fractionated and then combined (data not presented). This indicates that the dissimilar culture-dependent changes in 5′-N and APT′ase noted when low and high density cells were cultured individually was not induced by the gradient fractionation. Although not shown, neither low nor high density cells recovered at the end of the 3-day culture synthesized substantial amounts of PGE_2 (<5 ng/10^6 cells for each population, $p > 0.5$).

Effects of PGE_2 on culture-dependent changes in 5′-N and APT′ase. These studies indicate that differences in 5′-N and APT′ase do not serve as stable markers for Mϕ subpopulations. However, the data presented in Table I suggest that culture-dependent changes of 5′-N and APT′ase noted among unfractionated Mϕ cannot be accounted for by those occurring in each subpopulation. Low and high density fractions each represented approximately 50% of the recovered cells. Specific activities of 5′-N and APT′ase determined in the fresh, unfractionated Mϕ approximated the mean of that determined in the individual

TABLE I

Culture-dependent increases in Mϕ 5′-N and APT′ase

	Specific Activity[a]		Mean Increase
	Fresh Mϕ	Cultured Mϕ[b]	
A. 5′-N			
Unfractionated Mϕ	11 ± 6	440 ± 60	40
Low density Mϕ[c]	5.4 ± 1.3	192 ± 72	38
High density Mϕ[d]	19 ± 2.5	154 ± 65	8.1
B. APT′ase			
Unfractionated Mϕ	300 ± 50	510 ± 34	1.7
Low density Mϕ	347 ± 42	416 ± 42	1.2
High density Mϕ	235 ± 45	417[e]	1.8

[a] μg product/hr/mg cell protein; arithmetic mean ± S.E. for two to six experiments.

[b] Recovered after a 3-day incubation in HAS.

[c] Density gradient fraction 1 + 2 + 3.

[d] Density gradient fraction 4 + 5.

[e] Two experiments.

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fresh subpopulations. However, this same arithmetic relationship did not hold for cultured cells. One explanation for this is that interactions occur between unfractionated low and high density cells that can modulate culture-dependent changes in enzyme-specific activities. Such interactions would not occur when the subpopulations were cultured individually. A possible mediator of such an interaction could be PGE_2. This prostaglandin is synthesized by low but not high density cells. Moreover, it has been demonstrated that PGE_2 can modulate Mϕ metabolism (26–28).

As shown in Table II, PGE_2 is clearly capable of modulating culture-dependent increases in both enzymes occurring among whole, unfractionated monocytes. In four paired experiments, there was a mean 35-fold increase in the specific activity of 5′-N among whole monocytes cultured for 3 days in HAS. However, when the cells were cultured in the presence of indomethacin, a potent inhibitor of PGE_2 synthesis, 5′-N specific activities increased only 20-fold. (In the four paired experiments, the 5′-N levels among cells cultured in indomethacin were different from those determined among cells cultured in HAS, $p = 0.04$). Addition of exogenous PGE_2 in concentrations comparable to that actually synthesized by the monocytes (10^{-7} M; 24), restored the culture-dependent increases of 5′-N toward control values (5′-N levels among cells cultured in indomethacin and PGE_2 were not different from those among cells cultured in HAS, $p = 0.3$). PGE_2 also modulated culture-dependent increases in APT′ase. In four paired experiments, APT′ase increased by a mean of 1.5-fold among cells cultured in HAS, but 1.8-fold among cells cultured in indomethacin (the specific activity among cells cultured in indomethacin was different from that occurring among cells cultured in HAS, $p = 0.01$). Addition of exogenous PGE_2 restored the changes to control values (specific activities of APT′ase among cells cultured in indomethacin and PGE_2 *vs* those cultured in HAS were not different, $p = 0.3$). Thus, PGE_2 synthesized by Mϕ is required in part for the culture-dependent increases in 5′-N noted among unfractionated cells. In contrast, this same prostaglandin can blunt culture-dependent increases in APT′ase.

The data presented in Table III demonstrates that PGE_2 exerts similar effects on the fractionated cells. Addition of indomethacin to PGE_2 synthesizing low density cells blunted their culture-dependent increases in 5′-N but enhanced increases in APT′ase. Culture-dependent changes in both enzymes occurring among these low density cells was restored to control levels (cells cultured in HAS) by the addition of exogenous PGE_2. In contrast, indomethacin exerted relatively little effect on culture-dependent changes in 5′-N and APT′ase occurring among the non-PGE_2-synthesizing high density cells. Addition of PGE_2 to these cells resulted in markedly enhanced culture-dependent increases in 5′-N but blunted increases in APT′ase. Thus, PGE_2 modulates culture-dependent changes in 5′-N and APT′ase among Mϕ populations that do and those that do not synthesize this prostaglandin.

Note that when each population was cultured in equivalent concentrations of PGE_2, their relative differences in 5′-N and APT′ase were preserved. 5′-N levels among cultured low density Mϕ remained less than those among the high density population (194 *vs* 395 μg of product/hr/mg cell protein). Specific activities of APT′ase remained greater among the cultured low than among the cultured high density cells (338 *vs* 163 μg product/hr/mg cell protein). It should be emphasized that the recovery of viable cells from cultured low and high density fractions was not altered either by indomethacin or exogenous PGE_2.

TABLE II

Effect of PGE_2 on culture-dependent increases in Mϕ 5′-N and APT′ase among unfractionated Mϕ

		Specific Activity[a]			
			Mϕ cultured in[b]		
		Fresh Mϕ	HAS	Ind.	Ind. and PGE_2
I. 5′-N	Expt. 1	4.8	430	240	320
	2	20	790	470	650
	3	36	77	44	190
	4	21	160	60	172
Mean increase during culture:			35	20	28
II. APT′ase	Expt. 1	280	560	670	580
	2	280	360	380	360
	3	490	640	740	590
	4	360	510	600	500
Mean increase during culture:			1.5	1.8	1.5

[a] μg product/hr/mg of cell protein; data obtained with Mϕ from four different individuals.

[b] Cells were cultured for 3 days in either 10% heat-inactivated autologous serum (HAS), HAS containing 10^{-6} M indomethacin (Ind.), or HAS containing 10^{-6} M Ind. and 10^{-7} M PGE_2 (Ind. and PGE_2).

TABLE III

Effect of indomethacin and PGE_2 on culture-dependent changes in 5′-N and APT′ase among low and high density Mϕ

	Specific Activity[a]			
		Mϕ cultured in[b]		
	Fresh Mϕ	HAS	Ind.	Ind. and PGE_2
A. 5′-N				
Low density	3.5	239	139	194
High density	24.8	126	102	395
B. APT′ase				
Low density	316	354	436	338
High density	144	299	286	163

[a] μg product/hr/mg cell protein.

[b] Same conditions as used in Table II. Results represent the mean obtained in two to four experiments.

DISCUSSION

These studies demonstrate that: 1) gradient fractionated Mϕ can be broadly divided into two populations based on significant differences in their specific activities of 5′-N and APT′ase and by differences in their synthesis of PGE_2; 2) during short-term culture, increases in 5′-N and APT′ase occur among each population; and 3) these culture-dependent changes in enzyme-specific activities are modulated in part by PGE_2.

Although the data presented in Figure 1 suggest that biochemical heterogeneity exists among human Mϕ, the events that initiate this are not clear. It is unlikely that it was simply induced by the gradient fractionation. The specific activities of 5′-N and APT′ase determined in unfractionated fresh cells approximated the mean of those determined in the individual low and high density fractions (Table I; each of these fractions represented approximately 50% of the recovered cells). Moreover, incubation of low density cells in concentrations of BSA comparable to those present in the high density fractions, and vice versa, did not alter their relative differences in 5′-N, APT′ase, and PGE_2 synthesis (data not presented). We cannot rule out the possibility that early manipulations used to isolate

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the Mϕ (i.e., sedimentation over Ficoll-Hypaque, adherence to glass) induced alterations in the parameters measured here. However, we found that Mϕ comparably isolated and fractionated also demonstrated heterogeneity with regard to: 1) their display of Fc receptors and density of surface Ia determinants (29, H. Raff and J. Stobo, unpublished report), 2) their ability to support the concanavalin A-induced generation of suppressive T cells (14), and 3) the presence of a Mϕ specific-surface determinant detectable with a monoclonal antibody (H. Raff and J. Stobo, submitted for publication). Thus, if the differences in metabolic parameters measured here were induced by manipulations used for the cultivation and fractionation of Mϕ, then Mϕ are heterogenous in their response to these manipulations. Additionally, the changes induced during the isolation procedures were not limited to differences in metabolic parameters. For these reasons, it appears that the metabolic differences in Mϕ populations depicted here also exist as they circulate *in vivo*.

Density gradient fractionation of murine thymocytes and human myelocytes yields populations that differ in their display of certain differentiation markers (30, 31). Although both 5′-N and APT′ase have been used as markers of Mϕ differentiation, the most mature cells of the Mϕ line manifest high specific activities of *both* enzymes (13, 25). As indicated in Figure 1, none of the gradient fractions obtained here contained Mϕ with high specific activities of both 5′-N and APT′ase. Instead, fractions with the highest levels of 5′-N had the lowest levels of APT′ase and vice versa. Mϕ differentiation is also accompanied by a loss of peroxidase-positive, cytoplasmic granules (32). We could not find any obvious difference in the frequency of peroxidase-positive cells in each gradient fraction (H. Raff, J. Stobo, and D. Bainton, unpublished observations). For these reasons, it is unlikely that the Mϕ heterogeneity depicted here reflects the properties of cells in various stages of maturation.

A more likely possibility is that the differences in 5′-N, APT′ase, and PGE_2 synthesis noted among fractionated cells reflects the phenotypes of cells in various stages of activation. This process has been defined as a series of stepwise changes that culminate in enhanced Mϕ microbiocidal activity, and that is accompanied by characteristic changes in Mϕ morphology, expression of cell surface determinants, and biochemical activity (reviewed in 15–17). With regard to the specific parameters measured here, activation is accompanied by a decrease in the specific activity of 5′-N, an increase in the specific activity of APT′ase, and enhanced synthesis of PGE_2. Within this framework, low density Mϕ (fraction 1, 2, and 3) would represent the most and high density cells (fraction 4 + 5) the least activated. *In vivo* events that initiate this activation are conjectural. Although variation was noted in the absolute levels of 5′-N, APT′ase, and PGE_2 synthesis among fractionated cells from different individuals, the relative differences in these parameters among low *vs* high density cells from any given individual were remarkably constant. This suggests either that circulating Mϕ from different individuals are exposed *in vivo* to comparable amounts of activating influences or that a certain degree of activation is an intrinsic, programmed event.

Others have demonstrated that short-term culture of human peripheral blood Mϕ is accompanied by increases in their specific activities of both 5′-N and APT′ase (13, 25). The data presented here document this and also indicate that these culture-dependent changes in enzyme-specific activities are modulated in part by PGE_2. PGE_2 synthesis enhances culture-dependent increases in 5′-N but suppresses increases in APT′ase. This modulating effect of PGE_2 was confirmed by the studies showing that culture-dependent increases in 5′-N were greater among the PGE_2 synthesizing low density cells than among the non-PGE_2 synthesizing high density Mϕ. In contrast, culture-dependent increases in APT′ase were greater among the latter than among the former population. The significance of this observation is 2-fold. First, it demonstrates that PGE_2 synthesized by Mϕ can serve as a feedback mediator capable of modulating Mϕ metabolism. This is in keeping with other studies demonstrating that PGE_2 can modulate Mϕ phagocytosis, random migration, chemiluminescence, and synthesis of colony-stimulating factor (26–28). Second, it suggests that PGE_2 may play a role in modulating Mϕ differentiation. Short-term culture of Mϕ has been claimed to mimic *in vivo* differentiation as judged by changes in morphologic and histochemical criteria. The more mature cells recovered after the culture also manifest an increase in their specific activity of 5′-N and APT′ase, a change which we demonstrate is modulated by PGE_2. Mϕ may, through the synthesis of PGE_2, modulate the differentiation of other cell types (28, 33, 34). Thus, it is possible that PGE_2 also serves as a mediator by which they modulate their own differentiation. Since PGE_2 only altered culture-dependent increases in 5′-N and APT′ase by approximately 50%, this prostaglandin should be considered as a potential modulator rather than a requisite mediator for monocyte differentiation. It can be demonstrated that neither T cells nor B cells synthesize substantial amounts of PGE_2 (24). Thus, the PGE_2 that modulated Mϕ changes in 5′-N and APT′ase depicted here was synthesized by the Mϕ itself. Whether PGE_2 also modifies *in vivo* differentiation of Mϕ is not known.

Studies in animal models indicate that metabolic and functional heterogeneity exists among Mϕ. The studies presented here demonstrate the metabolic heterogeneity also exists among human peripheral blood Mϕ. Although we have not, in this report, investigated the functional properties of the Mϕ subpopulations, certain conclusions are implied. For example, the increased PGE_2 production by the PBMC of some patients with Hodgkin's disease might represent an increase in the frequency of Mϕ equivalent to the PGE_2 synthesizing low density cells demonstrated here (35). Moreover, the demonstrated immunoregulatory effects of PGE_2 on T cell reactivity might reflect the ability of this prostaglandin to effect the immunologic capabilities of Mϕ rather than to directly modulate effector or regulatory T cells (24). Further studies are needed to determine any role for prostaglandins in modulating the immunologic capabilities of Mϕ *in vivo*.

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REFERENCES

1. Rosenthal, A. S., and E. Shevach. 1973. Function of macrophages [illegible] recognition by guinea pig T lymphocytes. I. Requirement [illegible]ible macrophages and lymphocytes. J. Exp. Med. 138:1194.
2. Erb, P., and M. Feldman. 1975. The role of macrophages in the generation of T-helper cells. II. The genetic control of the macrophage-T-cell interaction for helper cell induction with soluble antigens. J. Exp. Med. 142:460.
3. Calderon, J., J.-M. Kiely, J. L. Lefko, and E. R. Unanue. 1975. The modulation of lymphocyte functions by molecules secreted by macrophages. I. Description and partial biochemical analysis. J.

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Exp. Med. 142:151.
4. Unanue, E. R. 1978. The regulation of lymphocyte functions by the macrophage. Immunol. Rev. 40:15.
5. Tadakuma, T., and C. W. Pierce. 1978. Mode of action of a soluble immune response suppressor (SIRS) produced by concanavalin A-activated spleen cells. J. Immunol. 120:481.
6. Ptak, W., M. Zembala, and R. K. Gershon. 1978. Intermediary role of macrophages in the passage of suppressor signals between T cell subsets. J. Exp. Med. 147:424.
7. Muschel, R. J., N. Rosen, and B. R. Bloom. 1977. Isolation of variants in phagocytosis of a macrophage-like continuous cell line. J. Exp. Med. 145:175.
8. Cowing, C., B. Schwartz, and H. Dickler. 1978. Macrophage populations differ in their expressions of Ia antigens. J. Immunol. 120: 378.
9. Heusser, C., C. Anderson, and H. Grey. 1977. Receptors for IgG: subclass specificity of receptors on different mouse cell types and the definition of two distinct receptors on a macrophage cell line. J. Exp. Med. 145:1316.
10. Snyderman, R., M. Pike, D. Fischer, and H. Koren. 1977. Biologic and biochemical activities of continuous macrophage cell lines P388D$_1$ and J774.1. J. Immunol. 119:2060.
11. Farr, A. G., W. J. Wechter, J. M. Kiely, and E. R. Unanue. 1979. Induction of cytocidal macrophages after *in vivo* interactions between *Listeria*-immune T cells and macrophages—role of H-2. J. Immunol. 122:2405.
12. Cowing, C., S. H. Pincus, D. H. Sachs, and H. B. Dickler. 1978. A subpopulation of adherent accessory cells bearing both I-A and I-E or C subregion antigens is required for antigen-specific murine T lymphocyte proliferation. J. Immunol. 121:1680.
13. Johnson, Jr., W. D., B. Mei, and Z. A. Cohn. 1977. The separation, long-term cultivation, and maturation of the human monocyte. J. Exp. Med. 146:1613.
14. Raff, H. V., K. Cochrum, and J. D. Stobo. 1978. Macrophage-T cell interactions in the Con A induction of human suppressive T cells. J. Immunol. 21:2311.
15. North, R. J. 1978. The concept of the activated macrophage. J. Immunol. 121:806.
16. Karnovsky, M. I., and J. K. Lazdins. 1978. Biochemical criteria for activated macrophages. J. Immunol. 121:809.
17. Cohn, Z. 1978. The activation of mononuclear phagocytes: fact, fancy, and future. J. Immunol. 121:813.
18. Stobo, J. D. 1977. Immunosuppression in man: suppression by macrophages can be mediated by interactions with regulatory T cells. J. Immunol. 119:918.
19. Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265.
20. Van der Zeijst, B. A. M., C. C. Sterwart, and S. Schlesinger. 1978. Proliferative capacity of mouse peritoneal macrophages *in vitro*. J. Exp. Med. 147:1253.
21. Allen, J. M., and J. Gockerman. 1964. Electrophoretic separation of multiple forms of particle associated acid phosphatase. Ann. N.Y. Acad. Sci. 121:616.
22. Goldyne, M. E., R. K. Winkelmann, and R. J. Ryan. 1973. Prostaglandin activity in human cutaneous inflammation: detection by radioimmunoassay. Prostaglandins 4:737.
23. Shaw, J. O., M. P. Printz, K. Hirabayshi, and P. Henson. 1978. Role of prostaglandin synthesis in rabbit platelet activation induced by basophil-derived platelet-activating factor. J. Immunol. 121:1939.
24. Goldyne, M. E., and J. D. Stobo. 1979. Synthesis of prostaglandins by monocyte subpopulations. Prostaglandins. In press.
25. Fischer, D., M. Van der Weyden, R. Snyderman, and W. Kelley. 1976. A role for adenosine deaminase in human monocyte activation. J. Clin. Invest. 58:399.
26. Wahl, L., C. Olsen, A. Sandberg, and S. Mergenhagen. 1977. Prostaglandin regulation of macrophage collagenase production. Proc. Natl. Acad. Sci. 74:4955.
27. Catarow, W., H. Cheung, and G. Sumdharudus. 1978. Effects of prostaglandins on the spreading, adhesion and migration of mouse peritoneal macrophages. Prostaglandins 16:39.
28. Kurland, J., H. Broxmeyer, L. Delus, R. Bockman, and M. Moore. 1978. Role for monocyte-macrophage-derived colony stimulative function and prostaglandin E in the positive and negative feedback control of myeloid stem proliferation. Blood 52:388.
29. Raff, H., L. Picker, M. E. Goldyne, and J. D. Stobo. 1979. Human monocyte heterogeneity. Clin. Res. 24:474A.
30. Stobo, J. D. 1972. Phytohemagglutinin and concanavalin A: probes for murine T cell activation and differentiation. Transplant. Rev. 11:60.
31. Winchester, R. J., G. D. Ross, C. I. Jarowski, C. Y. Wang, J. Harper and H. E. Broxmeyer. 1977. Expression of Ic-like antigen molecules on human granulocytes during early phases of differentiation. Proc. Natl. Acad. Sci. 74:4012.
32. Bainton, D., and D. Golde. 1978. Differentiation of macrophages from normal human bone marrow in liquid culture. J. Clin. Invest. 61:1555.
33. Gensa, D., M. Seitz, W. Gramer, G. Till, and K. Resch. 1978. The effects of phagocytosis, dextran sulfate, and cell damage of PGE_1 sensitivity and PGE_1 production of macrophages. J. Immunol. 120: 1187.
34. Weidemann, M., B. Peskar, K. Wrogemann, E. Th. Riethschal, H. Staudinger, and H. Rischer. 1978. Prostaglandin and thromboxane synthesis in a pure macrophage population and the inhibition, by E-type prostaglandins, of chemiluminescence. F.E.B.S. Lett. 89:136.
35. Goodwin, J. S., R. P. Messner, A. D. Bankhurst, G. T. Peake, J. H. Saiki, and R. C. Williams. 1977. Prostaglandin-producing suppressor cells in Hodgkin's disease. New Engl. J. Med. 297:963.

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