1,25-Dihydroxyvitamin D_3 Stimulates Phagocytosis but Suppresses HLA-DR and CD13 Antigen Expression in Human Mononuclear Phagocytes (43967)

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Abstract. **This study investigated the regulatory activity of 1,25-dihydroxyvitamin D_3 (1,25-$[OH]_2D_3$) on phagocytic cells obtained from normal human peripheral blood. Flow cytometric analysis enabled identification of two discrete populations of cells, one predominantly monocytes ("monocyte" gate) and one containing primarily lymphoid and other cell types ("lymphoid" gate). The monocyte-associated antigens CD13 and CD33 were highly expressed by cells in this monocyte gate and used to monitor this population. Following 5 days of culture, cells in the monocyte gate manifested high phagocytic activity as determined by ingestion of fluorescent carboxyl microspheres and exhibited high expression of class II HLA-DR products. 1,25-$(OH)_2D_3$ profoundly upregulated phagocytic activity while downregulating HLA-DR antigen expression on the cells in the monocyte gate. Moreover, 1,25-$(OH)_2D_3$ also reduced cell surface CD13 expression on the cells with low but not high phagocytic activity in this gate. Proportional activities by the 1,24-$(OH)_2D_3$ and 24,25-$(OH)_2D_3$ metabolites indicated the regulatory effects are likely mediated by the 1,25-$(OH)_2D_3$ receptor (VDR).**

Prostaglandin E_2 (PGE_2), a known modulator of monocyte/macrophage activity also markedly inhibited HLA-DR expression while enhancing the phagocytic activity of cells in the monocyte gate. In contrast to 1,25-$(OH)_2D_3$, PGE_2 clearly upregulated CD13 expression in cells with high phagocytic activity. Since indomethacin, an inhibitor of PGE_2 synthesis, failed to reverse the 1,25-$(OH)_2D_3$ induced inhibitory effect on HLA-DR expression, this effect is apparently not mediated through endogenous PGE_2 synthesis. Based on these findings we speculate that 1,25-$(OH)_2D_3$ may be capable of acting as both an upregulating agent during natural immunity via the enhancement of phagocytosis by monocyte/macrophage populations and as a "downregulator" during acquired immune responses via an inhibitory effect on MHC class II antigen expression by professional antigen-presenting cells. [P.S.E.B.M. 1996, Vol 211]

The biologically active metabolite of vitamin D_3, 1,25-dihydroxyvitamin D_3 (1,25-$[OH]_2D_3$), is a calcium-regulating hormone. However, recent works have demonstrated that specific cytosol or nuclear receptors for 1,25-$(OH)_2D_3$ (VDR) exist in a variety of tissues other than classical target tissues (i.e. bone, intestine, kidney) such as the immune system (1–4). Additionally, 1,25-$(OH)_2D_3$ was shown to have immunoregulatory abilities including inhibition of immunoglobulin secretion by B cells and the production of interleukin-2 (IL-2), IL-2R, granulocyte-macrophage colony–stimulating factor, and γ-interferon (IFN-γ) by stimulating T cells, as well as inhibition of accessory cell and antigen-presenting cell (APC) activity (5–10). In contrast to such inhibitory effects, 1,25-$(OH)_2D_3$ was reported to enhance several monocyte and macrophage functions *in vitro* including phago-

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cytosis, killing of mycobacteria, production of hydrogen peroxide, and the expression of heat shock proteins (11–13).

Tsoukas *et al.* demonstrated that pre-treatment of human normal monocytes with 1,25-$(OH)_2D_3$ clearly inhibited both the production of IL-1 and APC function by these cells in subsequent culture with T cells (14). With respect to MHC expression, we recently reported that 1,25-$(OH)_2D_3$ profoundly but not completely antagonized IFN-γ–induced expression of class II MHC antigens in resident endocrine tissue populations such as rat thyroid follicular and mouse testicular Leydig cells and human peripheral monocytes (15, 16). The purpose of the present study was to begin to examine the effect of vitamin D_3 metabolites on the phenotype and function of human peripheral blood phagocytic cells presumably representing monocyte/macrophage lineage cells. The results of the present study demonstrated that 1,25-$(OH)_2D_3$ metabolites have multiple effects on this population involving downregulation of monocyte class II HLA-DR and CD13 products in contrast to upregulating their phagocytic activity. These effects appear to be mediated via interaction with VDR in human peripheral blood monocytes.

Materials and Methods

Cell Preparations. Heparinized peripheral blood from healthy volunteers or buffy coats from the American Red Cross (Miami, FL) were separated by Ficoll Hypaque density gradient centrifugation. The cells were resuspended in RPMI-1640 medium supplemented with fetal calf serum (15%) and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air.

Reagents. Prostaglandin E_2 (PGE_2) was purchased from Sigma Chemical Co. (St. Louis, MO). Vitamin D_3 metabolites; 1,25-$(OH)_2D_3$; 24,25-$(OH)_2D_3$; and 1,24-$(OH)_2D_3$ were kindly provided by Y. Uotani, Teijin Ltd. (Tokyo, Japan). All metabolites and PGE_2 were dissolved in absolute ethanol, and the final concentrations of ethanol were approximately 0.1%–1%, equivalent to the level contained in 100–1000 n*M* of vitamin D_3 metabolites or PGE_2. Control experiments using various concentrations of ethanol demonstrated that 0%–1% ethanol had no detectable effect on HLA-DR and CD13 expression or phagocytic activity on peripheral blood monocytes.

Fluorescent Analysis for Phagocytosis. As previously reported (17), fluorescent carboxyl microspheres (1.75 μm; Polysciences, Warrington, PA) have been used to assess phagocytosis. The microspheres (10^7 in 100 μl of culture medium) were added to the cultures for 2 hr, at which time the cells were harvested (adherent cells were detached by treatment with trypsin-EDTA for 5 min at 37°C), centrifuged, and resuspended in fluorescence-activated cell sorter (FACS) medium (phosphate-buffered saline containing 0.1% bovine serum albumin and 0.05% NaN_3 supplemented with 1% paraformaldehyde) unless further fluorescence analysis was performed.

Monoclonal Antibodies. The monoclonal antibodies used in this study were kindly provided by Dr. Phillip Ruiz, Department of Pathology, University of Miami (Miami, FL): I3, phycoerythrin (PE)–conjugated anti-HLA-DR (mouse IgG_{2a}, Coulter Corp., Hialeah, FL); My7, PE-conjugated anti-CD13 (mouse IgG_1, Coulter); My9, fluorescein isothiocyanate (FITC)–conjugated anti-CD33 (mouse IgG_{2b}, Coulter).

Fluorescent Analysis of Monoclonal Antibody Staining. After phagocytosis, cells were harvested, washed once in FACS media, and all samples were incubated for 45 min at 4°C with heat-aggregated human IgG (1 mg/ml) to block nonspecific binding of the monoclonal antibodies to Fc receptors. Control samples were incubated with an isotype-matched irrelevant antibody (i.e., for anti-HLA-DR [mouse IgG_{2a}; 34-2-12, anti-H-$2D^d$], for anti-My 7 [mouse IgG_1; W3/25 anti-rat framework class I MHC]). Experimental samples were stained with PE- and FITC-conjugated monoclonal antibodies described above. Cells stained with PE-conjugated mouse IgG (Coulter) were included in each group and used as negative controls. All samples (3000 cells/sample) were analyzed on a FACS (FACScan; Becton Dickinson, Mountain View, CA). Live gates based on forward angle and side angle light scatter were used to eliminate dead cells and debris. Data are represented as mean fluorescence intensity (MFI) and the percentage of positively staining cells.

Results

Identification of Human Peripheral Blood Phagocytic Cells by Flow Cytometry. Using forward (indicative of cell size) and side (indicative of cell density) scatter two distinct cell populations in peripheral blood mononuclear cells (PBMC) following ficoll hypaque separation can be readily identified by flow cytometric analysis (Fig. 1A). One population predominantly contains human monocytes with few lymphoid cells (referred to as the "monocyte gate"), and the second population predominantly contains lymphoid cells (referred to as the "lymphocyte gate"). Seventy to ninety percent of the cells in the monocyte gate were found to express monocyte/macrophage–associated markers (CD33), while less than 5% of the cells in the lymphocyte gate expressed these markers (data not shown). Initially, we examined the phagocytic activity of the cells in the monocyte gate. To quantitatively analyze this activity, 1.75-μm–diameter fluorescent carboxyl microspheres were used as targets for phagocytosis. Figure 1B shows a representative fluorescent pattern after a 2-hr incubation with fluo-

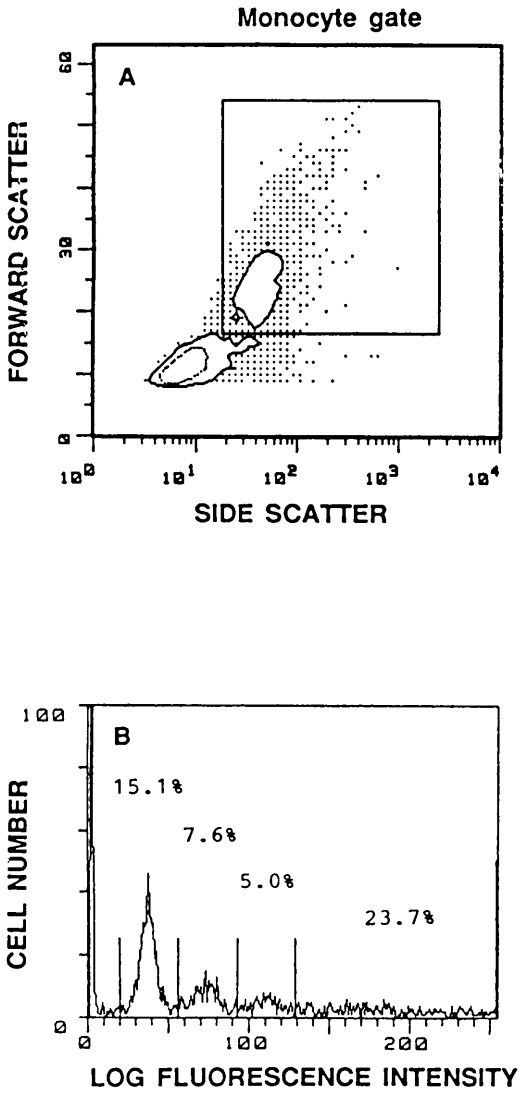

Figure 1. (A) Forward and side angle light scatter of human peripheral blood mononuclear cells. Cells within Box 1 are predominantly monocytes and this gate is referred to as the "monocyte gate". Box 2 contains predominantly lymphoid cells (i.e., the "lymphocyte gate"). (B) Flow cytometric analysis of peripheral blood mononuclear cells phagocytosing increasing numbers of microspheres. PBMC were incubated for 2 hr with 10^7 fluorescent carboxyl microspheres, harvested, and fixed as described in Materials and Methods, and subsequently analyzed by flow cytometry. The percentages (15.1, 7.6, 5.0, and 23.7) shown refer to the percentage of cells internalizing one, two, three, or more microspheres respectively.

rescent microspheres. The first, second, third, and fourth peaks represent cells internalizing one (15.1%), two (7.6%), three (5%), or four or more (23.7%) microspheres, respectively. More than half of the cells in the monocyte gate were observed to phagocytose one or more microspheres within 2 hr of exposure. In contrast, less than 1% of the cells in the lymphocyte gate exhibited marginally detectable phagocytic activity (data not shown).

Effects of $1,25\text{-}(OH)_2D_3$ Metabolites and PGE_2 on HLA-DR Expression and Phagocytic Functions in Peripheral Blood Monocytes. As noted above, cells in the "lymphoid" gate were found to phagocytose few microspheres, whereas, a large component of cells (40%–60%) in the monocyte gate were phagocytic and thus considered to represent peripheral blood phagocytes. As previously reported, $1,25\text{-}(OH)_2D_3$ metabolites can regulate expression of class II MHC expression in a variety of cell types (15, 16). We therefore examined the effects of $1,25\text{-}(OH)_2D_3$ metabolites on HLA-DR in peripheral phagocytes in the monocyte gate (Table I, one of three independent experiments). HLA-DR antigens were strongly expressed by most of the cultured cells within the monocyte gate both with respect to percentage positive (Group 1: 81%) or MFI (i.e., the HLA-DR antigen density per cell) (Group 1: MFI = 595). Even within the monocyte gate the phagocytosing cells expressed a higher level of HLA-DR antigen expression than the nonphagocytosing cells (Group 1: MFI = 683 vs 536). The cells that phagocytosed one or two microspheres consistently expressed slightly greater levels of HLA-DR antigens than the cells that phagocytosed three or more microspheres in this gate (Group 1: MFI = 737 vs 637).

Compared with no treatment, treatment with $1,25\text{-}(OH)_2D_3$ was found to markedly reduce HLA-DR expression in both nonphagocytosing cells (MFI: 536 to 274) and cells phagocytosing one or two microspheres (MFI: 737 to 131), or three or more microspheres (MFI: 637 to 31). This inhibitory effect of $1,25\text{-}(OH)_2D_3$ on class II was dose dependent (i.e., 0.1–1000 n*M*) and was not observed with respect to class I expression (data not shown and Refs. 9, 10, and 16). Notably, $1,25\text{-}(OH)_2D_3$ metabolites $1,24\text{-}(OH)_2D_3$ and $24,25\text{-}(OH)_2D_3$ manifested inhibitory effects on HLA-DR expression on these phagocytic cells. Moreover, $24,25\text{-}(OH)_2D_3$, which has 500- to 1000-fold lower binding affinity for the vitamin D receptor (VDR) than $1,25\text{-}(OH)_2D_3$ or $1,24\text{-}(OH)_2D_3$, was clearly less effective than $1,25\text{-}(OH)_2D_3$ or $1,24\text{-}(OH)_2D_3$ suggesting that the inhibitory effects were mediated by VDR (Table I, right panel). This downregulation of HLA-DR expression by $1,25\text{-}(OH)_2D_3$ metabolites was most apparent in the cells phagocytosing three or more microspheres versus those cells phagocytosing zero to two microspheres (Group 1 vs 2: three or more microsphere, 95.1% inhibition; one or two microspheres, 82.2% inhibition; zero microspheres, 48.9% inhibition).

In contrast to their inhibitory effects on HLA-DR, $1,25\text{-}(OH)_2D_3$ metabolites were found to enhance the phagocytic function of the cells in the monocyte gate (Table I). The numbers of viable cells and the actual percentage of the cells in the monocyte gate between untreated and $1,25\text{-}(OH)_2D_3$–treated groups were not significantly different (data not shown). The enhancing effects of the $1,25\text{-}(OH)_2D_3$ metabolites were apparent as a result of the overall increase in the numbers of

Table I. Effect of 1,25-$(OH)_2D_3$ Analogs and PGE_2 on Phagocytic Activity and HLA-DR Expression on Phagocytes

Group	Treatment[a]	% Phagocytosis[b]				HLA-DR expression (MFI) on phagocytosing cells[c]				
		0	≥1	1 or 2	≥3	Total	0	≥1	1 or 2	≥3
1	Untreated	60	40	19	22	595 (81%)	536	683	737	637
2	$1,25D_3$ (100 nM)	28	72	15	57	114 (37)	274	52	131	31
3	$1,24D_3$ (100 nM)	28	72	16	56	115 (39)	246	64	115	50
4	$24,25D_3$ (100 nM)	55	45	19	26	529 (78)	484	585	626	556
5	$24,25D_3$ (1000 nM)	33	67	15	53	223 (53)	309	180	298	147
6	PGE_2 (1000 nM)	22	78	11	67	134 (38)	295	88	201	70

[a] PBM cells were cultured for 5 days in the absence (untreated) or presence of vitamin D_3 analogs or PGE_2.
[b] After 5-day treatments, cells were incubated with fluorescent microspheres for 2 h and evaluated for phagocytosis and HLA-DR expression in the monocyte gate. Data are shown in terms of percentage of the cells phagocytosing 0, ≥1, 1 or 2, or ≥3 microspheres.
[c] Data are presented in terms of mean fluorescence intensity of the population. Number in parenthesis represents the percentage positive cells determined by analytical gates established using PE-conjugated normal Ig.

cells that phagocytosed three or more microspheres (Group 1 vs 2: one or two microspheres, 19% vs 15%; three or more microspheres, 22% vs 57%). Therefore, 1,25-$(OH)_2D_3$ metabolite treatments not only markedly reduced the percentage of nonphagocytosing cells within the first 2 hr (60% → 28%) but also more than doubled the number of phagocytes in the monocyte gate exhibiting a high level of phagocytic activity and coincidently expressing a low level of HLA-DR antigens. Ten-fold higher concentrations of 24,25-$(OH)_2D_3$ were required (Group 5) for effects equivalent to 1,25-$(OH)_2D_3$ or 1,24-$(OH)_2D_3$ (Table I, left panel).

PGE_2 has been well established as an agent that can downregulate class II MHC expression and upregulate phagocytic function in monocyte/macrophage lineage cells (18). In the present study, PGE_2 also downregulated HLA-DR and upregulated phagocytic function in the peripheral blood population being examined (Table I, Group 6; Table II, Group 4). Thus, the present findings demonstrate that 1,25-$(OH)_2D_3$ metabolites exhibited effects on HLA-DR expression and phagocytosis commensurate with PGE_2.

Effect of Inhibition of the Arachidonic Acid Pathway on 1,25-$(OH)_2D_3$ Downregulation of HLA-DR Expression in Peripheral Blood Monocytes. Since monocytes treated with 1,25-$(OH)_2D_3$ may release increased amounts of PGE_2 (19), the products of the arachidonic acid pathway could be responsible for mediating 1,25-$(OH)_2D_3$–induced inhibition of HLA-DR expression. CD33 (detected by mAb MY9) is reportedly expressed by peripheral blood monocyte/macrophage lineage cells, but not by granulocytes, erythrocytes, lymphocytes, or platelets (20). To evaluate monocyte/macrophage cells more precisely, we examined the effect of indomethacin, a known inhibitor of the arachidonic acid pathway and PGE_2 synthesis on HLA-DR expression of $CD33^+$ cells (83% of the cells in the monocyte gate) in the monocyte gate. At all concentrations tested (0.01–10,000 n*M*), indomethacin failed to reverse the 1,25-$(OH)_2D_3$–induced inhibition of HLA-DR expression in these peripheral blood $CD33^+$ monocytes (Fig. 2).

Effect of 1,25-$(OH)_2D_3$ and PGE_2 on CD13 Expression in Peripheral Blood Phagocytic Monocytes. Although their function is not yet known, CD13 antigens (detected by mAb MY7) are reportedly expressed by monocyte/macrophage lineage cells and therefore are expressed by cells exclusively in the monocyte gate (21). To determine if expression of these antigens was altered in nonphagocytosing or phagocytosing peripheral blood mononuclear cells, we examined the effect of 1,25-$(OH)_2D_3$ on the expression of CD13 by cells within the monocyte gate. The experiment (representative of three performed) indicated

Table II. Effect of Vitamin D_3 Analogue and PGE2 on CD13 Expression on Phagocytes

Group	Treatment[a]	% Phagocytosis[b]				CD13 expression (MFI) on phagocytosing cells[c]			
		0	≥1	1 or 2	≥3	Total	0	1 or 2	≥3
1	Untreated	58	42	19	23	233 (78%)	170	205	414
2	$1,25D_3$ (100 nM)	22	78	18	61	224 (60)	29	27	350
3	$1,24D_3$ (100 nM)	22	78	18	59	250 (62)	30	31	401
4	PGE_2	18	82	12	71	752 (81)	55	98	1034

[a] PBM cells were cultured for 5 days in the absence (untreated) or presence of $1,25D_3$, $1,24D_3$, or PGE_2.
[b] After 5-day treatments, cells were incubated with fluorescent microspheres for 2 h and then stained with PE–anti-CD13 mAb (My7). Data are shown in terms of percentage of the cells in the monocyte gate phagocytosing 0, ≥1, 1 or 2, or ≥3 microspheres.
[c] Data are presented in terms of mean fluorescence intensity of the population. Number in parenthesis represents the percentage positive cells determined by analytical gates set using PE-conjugated normal Ig.

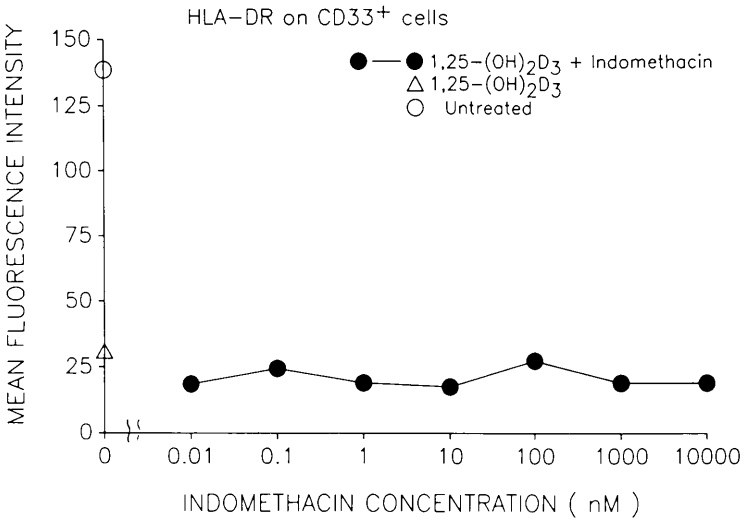

Figure 2. Effect of indomethacin on the ability of 1,25-$(OH)_2D_3$ to inhibit HLA-DR class antigen expression on $CD33^+$ cells. PBMC were cultured for 4 days in medium alone (○), or containing 100 n*M* 1,25-$(OH)_2D_3$ in the absence (△) or presence of varying concentrations of indomethacin (●). $CD33^+$ cells in the monocyte gate were analyzed for HLA-DR expression.

that after incubation of PBM cells for 5 days in normal culture medium, 78% of the cells in the monocyte gate expressed CD13 antigen (Table II). The most actively phagocytic cells among them (i.e., those that phagocytosed three or more microspheres) expressed augmented levels of CD13 antigens (greater than twice the levels) compared with $CD13^+$ cells with less phagocytic activity (zero to two microspheres) with respect to MFI (Group 1: three or more microspheres, 414 vs. one or two microspheres, 205 and/or zero microsphere, 170).

Interestingly, exposure to 1,25-$(OH)_2D_3$ metabolites for 5 days (Table II) resulted in downregulation of CD13 antigen (CD33 antigen expression was not affected by this treatment, personal observation, data not shown, N.T., R.B.L.) expression by the cells within the monocyte gate, primarily on the cells with low phagocytic activity (Group 1 vs 2: zero microsphere, 170 to 29; one or two microspheres: 205 to 27). Thus, 1,25-$(OH)_2D_3$ treatment was again found to mediate opposing effects, enhancing the phagocytic activity of cells in the monocyte gate while diminishing (similar to HLA-DR) the expression of CD13 antigen in the cells exhibiting low levels of phagocytic activity. However, in contrast to the HLA-DR results, the cells with high phagocytic activity were found to express relatively high levels of CD13 antigen even after treatment with 1,25-$(OH)_2D_3$ (three or more microspheres, 414 to 350).

PGE_2 was also found to alter expression of CD13. Similar to its effects (and those of 1,25-$[OH_2D_3]$ on HLA-DR, PGE_2 downregulated the expression of CD13 antigen in cells with low phagocytic activity (Table II). However, in contrast to 1,25-$(OH)_2D_3$, PGE_2 clearly upregulated the expression of CD13 in the majority of cells examined, including those exhibiting high levels of phagocytic activity. These findings indicate that the effects of these agents on phagocytic cells are not completely equivalent.

Discussion

The results of the present investigation of human peripheral blood mononuclear cells demonstrated the following effects by 1,25$(OH)_2D_3$ metabolites: (i) marked enhancement of phagocytosis by monocytes/macrophages; (ii) concurrent with this enhancement, a strong downregulation of HLA-DR expression; and (iii) a less marked downregulating activity on CD13 expression. Similar effects were obtained with those previously reported for endogenous PGE_2 with respect to phagocytosis and HLA-DR expression by monocytes/macrophages (18, 22, 23). However, in contrast to 1,25$(OH)_2D_3$, PGE_2 augmented CD13 expression on actively phagocytosing cells. Thus, while mediating similar effects, these two regulators likely invoke at least some differing signaling pathways.

Similar to other steroids and their receptors, VDR are considered to mediate most of the biological actions of 1,25-$(OH)_2D_3$ (3). The binding affinities of several 1,25-$(OH)_2D_3$ metabolites are known to vary from equivalent (1,24-$[OH]_2D_3$) to approximately 500- to 1000-fold lower (24,25-$[OH]_2D_3$) than 1,25-$(OH)_2D_3$. The results in the present study found the effect of these metabolites on phagocytosis, HLA-DR and CD13 expression in phagocytic cells were proportional to their binding affinities, suggesting that the 1,25-$(OH)_2D_3$ interaction with the VDR plays a crucial role in these effects.

We and other investigators have recently shown the regulatory activity of 1,25-$(OH)_2D_3$ on MHC class II antigen expression in a variety of cell types, including mouse testicular Leydig and rat thyroid cell lines (15), human melanoma cell line (24), human T and B lymphocytes (Tokuda, Levy, personal observation),

human keratinocytes (25), human corneal epithelial cells (Ohashi, Mano, personal communication), and mouse and human monocyte/macrophage tumorigenic cell lines (26). Since, 1,25-$(OH)_2D_3$ has been found to downregulate the induction of MHC class II expression in most of the cell types except for monocyte/macrophage tumorigenic cell lines (26), we were interested in determining the effect of 1,25-$(OH)_2D_3$ on class II induction in normal human monocyte/macrophage lineage cells. The previous (16) and present results clearly indicate that 1,25-$(OH)_2D_3$ downregulates class II expression on the human peripheral blood phagocytic and $CD33^+$ cells. The inhibitory effects of 1,25-$(OH)_2D_3$ were observed at the concentration of $\geq$0.1 n*M*, within the range of normal blood levels (0.09 n*M* [27]).

In contrast to this inhibitory effect on MHC class II induction, 1,25-$(OH)_2D_3$ upregulated the phagocytic activity of peripheral blood monocyte/macrophage cells. Moreover, MHC class II expression in cells exhibiting high phagocytic activity was found to be most sensitive to 1,25-$(OH)_2D_3$ inhibition. Consequently, 1,25-$(OH)_2D_3$ treatment increased the number of cells with high phagocytic activity and low class II expression. It is therefore possible that 1,25-$(OH)_2D_3$ could induce immature monocytes to differentiate into effective professional phagocytes at the "potential expense" of immune antigen-presenting cell function.

CD13 antigen (i.e., aminopeptidase N [28]) is known to be expressed primarily by granulocytes and monocyte/macrophage cells (21, 29), two populations that mediate phagocytic activity. However, little is known concerning the function of this antigen. We found that approximately 80% of untreated cultured cells in the monocyte gate expressed CD13 antigens following 5 days of culture (Table II). Interestingly, the amount of this antigen on the cell surface appeared to correlate with the level of phagocytic activity. CD13 antigen expression was affected (i.e., reduced by) 1,25-$(OH)_2D_3$, but, in contrast to MHC class II expression, CD13 downregulation was primarily observed in the cells with no or low phagocytic activity. O'Connell *et al.* have recently shown that mAb to CD13 antigen caused a dose-dependent reduction in C3bi binding to neutrophils and, moreover, that CD13 may function as a signal transduction molecule (30). Since the present results found a correlation between phagocytic activity and the amount of this antigen per cell in the absence of 1,25-$(OH)_2D_3$, it is possible that CD13 may be a molecule involved in phagocytosis. Further studies are necessary to address this hypothesis.

Recently, a number of biological molecules, such as PGE_2 (18), IFN-α/β (31, 32), α-fetoprotein (33), glucocorticoids (31, 32), and lipopolysaccharide (34), have been found to inhibit MHC class II expression in murine or human macrophages. Some of these inhibitory effects appear to be mediated by the production of PGE_2 (35). Since PGE_2 is one of the molecules produced by monocyte/macrophage lineage cells (36) and 1,25-$(OH)_2D_3$ may stimulate monocytes to release increased amounts of PGE_2 (19), it was possible that the 1,25-$(OH)_2D_3$ effects detected on the human phagocytic cells studied here involved the production of PGE_2. However, indomethacin (0.01–10,000 n*M*) failed to reverse the inhibitory effect of 1,25-$(OH)_2D_3$ on HLA-DR class II expression in $CD33^+$ cells. Since (i) the effect of PGE_2 on CD13 expression was somewhat different from 1,25-$(OH)_2D_3$ in this study and (ii) our previous studies demonstrated 1,25-$(OH)_2D_3$ but not PGE_2 could inhibit IFN-γ–induced MHC class II expression in mouse testicular Leydig cells (15), the results suggest that the effect of 1,25-$(OH)_2D_3$ on class II expression is distinct from that of PGE_2 and does not involve the production of endogenous PGE.

Based on the findings to date concerning 1,25-$(OH)_2D_3$, we speculate that these metabolites could participate as upregulators during natural defense reactions by augmenting phagocytosis by monocyte/macrophage cells. In contrast, since MHC class II antigen expression on APC is essential for antigen recognition by $CD4^+$ T lymphocytes, and the ability of APC to present conventional antigens to T cells has been shown to correlate with the amount of class II antigen on the cell surface of APC (37–39), 1,25-$(OH)_2D_3$ may also contribute to downregulating immune responses through the inhibition of antigen-presenting capabilities of professional (monocyte/macrophage) as well as nonprofessional APCs.

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