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1,25-dihydroxyvitamin D_3 down-regulation of HLA-DR on human peripheral blood monocytes

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SUMMARY

The regulatory activity of 1,25-dihydoxyvitamin D_3 (1,25-$(OH)_2D_3$) on human leucocyte antigen (HLA)-DR (MHC class II) antigen expression in monocytes from normal human peripheral blood was examined. Using forward light and side scatter by flow cytometry most cells within the discrete monocyte area expressed high levels of HLA-DR antigens following 4-day culture in medium alone (culture-enhanced HLA-DR) and expression was further up-regulated in the presence of interferon-gamma (IFN-γ) (IFN-γ-enhanced HLA-DR). Treatment with 1,25-$(OH)_2D_3$ markedly inhibited both culture and IFN-γ-enhanced HLA-DR but not HLA-ABC (MHC class I). This 1,25-$(OH)_2D_3$ inhibition was as effective as PGE_2 and hydrocortisone. To ascertain if HLA-DR was specifically down-regulated on monocytes, the effect of vitamin D_3 analogues in $CD33^+$ cells was examined. Incubation of the $CD33^+$ cells with 1,25-$(OH)_2D_3$, 24-25-$(OH)_2D_3$ and 25-OHD_3 resulted in dose-dependent inhibition of culture-enhanced HLA-DR parallelling the vitamin D_3-receptor affinities of these compounds. Northern analysis also demonstrated that 1,25-$(OH)_2D_3$ treatment markedly decreased both expression of culture-enhanced and IFN-γ-enhanced HLA-DRβ chain messanger RNA (mRNA) in monocyte-enriched populations. In total, our findings are consistent with the proposal that vitamin D_3 analogues can contribute to down-regulating immune responses as a consequence of inhibiting class II expression.

INTRODUCTION

Expression of class II major histocompatibility complex (MHC) antigens on antigen-presenting cells (APC) is required for antigen presentation to $CD4^+$ helper T cells and there is evidence for the quantitative relationship between class II MHC antigen expression and APC function.[1-3] Within the past several years a number of molecules including interferon-gamma (IFN-γ),[4] recombinant interleukin-4 (rIL4-4),[5] and tumour necrosis factor (TNF)[6] have been reported to augment MHC class II expression on a variety of cell types. Other agents, including IFN-α/β,[7] α-fetoprotein,[8] glucocorticoids,[7,9] prostaglandin E (PGE) series,[10] and lipopolysaccharide[11] have been found to inhibit class II expression. Notably, the majority of the inhibition studies have focused on monocyte and macrophage cell populations.

The seco-steroid 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$), the most biologically active form of vitamin D_3, has been found to effect a number of immune responses.[12] In addition to direct inhibitory effects on cytokine production by T cells, B cells and macrophages,[13-16] 1,25-$(OH)_2D_3$ has recently been shown to regulate class II MHC expression in a number of cell populations. Interestingly, 1,25-$(OH)_2D_3$ has been reported to both augment and diminish class II expression. For example, 1,25-$(OH)_2D_3$ has been shown to antagonize IFN-γ induction of class II in mouse testicular Leydig and rat thyroid follicular cell lines in our laboratory[17] and decrease constitutive expression of class II in a human melanoma cell line.[18] In contrast, 1,25-$(OH)_2D_3$ was previously reported to enhance IFN-γ induction of class II in the mouse monocytic tumour cell line WEHI-3.[19]

In the present study, we examined the effect of vitamin D_3 analogues, alone and together with IFN-γ on HLA-DR class II antigen expression in normal human peripheral blood monocytes. In contrast to the reported enhancement of class II on myeloid/monocytic tumour cell lines, 1,25-$(OH)_2D_3$ was found to profoundly down-regulate HLA-DR expression in human peripheral blood monocytes at both the protein and messanger RNA (mRNA) levels.

MATERIALS AND METHODS

Cell preparation

Buffy coats from the American Red Cross (Miami, FL) or heparinized venous blood from healthy donors were used as a

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source for human peripheral blood mononuclear cells (PBMC). PBMC were separated by Ficoll Hypaque (Sigma, St Louis, MO) density gradient centrifugation. Cells were resuspended in RPMI-1640 medium supplemented with foetal calf serum (FCS, 15%) or autologous serum (15%).

Reagents

$1,25\text{-}(OH)_2D_3$, $24,25\text{-}(OH)_2D_3$ and $25\text{-}OHD_3$ dissolved in absolute ethanol were generously provided by Y. Uotani, Teijin Ltd (Tokyo, Japan). The final ethanol concentration in wells receiving these samples was usually less than 0·1% (i.e. the final ethanol concentration in wells containing vitamin D_3 analogues at 100 nM). Recombinant human IFN-γ (Collaborative Research Inc., Bedford, MA) and prostaglandin E_2 (PGE_2), hydrocortisone and T_3 (Sigma) were added to desired concentrations.

Monoclonal antibodies

The following monoclonal antibodies (mAb) were utilized: W6/32, anti-HLA-A,B,C (mouse IgG2a); I3, phycoerythrin (PE)-conjugated anti-HLA-DR (mouse IgG2a, Coulter Corp., Hialeah, FL); MO2, PE-conjugated anti-CD14 (mouse IgM, Coulter Corp.); My9, fluorescein isothiocyanate (FITC)-conjugated anti-CD33 (mouse IgG2b, Coulter Corp.); B4, FITC-conjugated anti-CD19 (mouse IgG_1, Coulter Corp.); and T11, PE-conjugated anti-CD2 (mouse IgG1, Coulter Corp.), kindly provided by Dr P. Ruiz, University of Miami School of Medicine, FL.

Immunofluorescent staining and analysis

Cultured non-adherent floating cells and adherent (treatment with trypsin-EDTA) PBMC were washed in phosphate-buffered saline (PBS) containing 0·1% bovine serum albumin and 0·05% NaN_3. Cells (5×10^5 to 1×10^6) were then preincubated for 45 min at 4° with heat-aggregated human IgG (1 mg/ml) plus isotype matched immunoglobulins: mouse IgG2a: 34-2-12, anti-H-$2D^d$, 1:40 dilution of ascites fluid; mouse IgG2b: 10-2-16, anti-I-A^k, 1:40 ascites; or mouse IgM: HO2.2, anti-Lyt-2, neat culture supernatant in an effort to block non-specific binding of the mAb to Fc receptors. Subsequently, cells were directly stained with FITC- or PE-conjugated mAb or indirectly stained with W632 mAb followed by FITC-conjugated goat Ab to mouse IgG (FITC-GAM, IgG $F(ab')_2$ fragments (Organon Teknika Corp., Durham, NC).

Cells incubated with FITC-GAM (i.e. second Ab) alone for indirect staining or the mixture of FITC-conjugated mouse IgG and IgM and PE-conjugated mouse IgG for direct staining were included in each group and all samples were analysed on a fluorescence-activated cell sorter (FACScan, Becton Dickinson, Mountain View, CA). Data are presented as mean fluorescence intensity (MFI) and percentage of positive cells based on background staining of 1–2% (second antibody alone).

RNA preparation

In order to enrich for monocytes, PBMC (4×10^6/ml) were resuspended in RPMI-1640 supplemented with human AB serum (5%) and cultured in plastic Petric dishes overnight. Non-adherent cells were removed by repeated washes with warm medium. Tightly adherent cells were detached by treatment with trypsin-EDTA and cultured in RPMI-1640 supplemented with FCS (15%) in the presence of $1,25\text{-}(OH)_2D_3$ alone or together with IFN-γ for another 2 days. Total cellular RNA from the cells recovered by trypsinization was extracted by the guanidinium isothiocyanate method.[20] RNA was quantitated by UV absorbance at 260 nm. RNA samples were denatured at 70° for 5 min, size separated by electrophoresis in a 2·2 M formaldehyde/1% agarose gel and transferred to a nylon membrane filter (Bio-Rad Laboratories, Richmond, CA).

Northern blot analysis

The HLA-DR β-chain cDNA probe [21] was radiolabelled by the hexamer priming method of Feinberg & Vogelstein[22] and hybridized to membrane-bound RNA. After overnight hybridization at 42° in a solution containing 50% formamide, 1 M NaCl, 10 × Denhardt's solution, 50 mM Tris-HCl (pH 7·5), 1% $Na_4P_2O_7$, 1% sodium dodecyl sulphate (SDS), and 150 μg/ml of sheared and denatured salmon sperm DNA, the filters were washed twice with 2 × SSC (1 × SSC is 15 mM sodium citrate, 150 mM NaCl, pH 7·0), 0·1% SDS at 50°, and then twice with 0·1 × SSC, 0·1% SDS at 50°. The filters were then exposed to Kodak film with intensifying screen at −70°. Autoradiograms of films exposed for differing times were scanned in a laser densitometer in order to determine the amount of specific hybridization.

RESULTS

Inhibitory effect of $1,25\text{-}(OH)_2D_3$ on HLA-DR antigen expression of cultured human monocytes

Human monocyte/macrophages amongst PBMC are predominantly localized within a discrete area as analysed using forward light (i.e. cell size) and side (i.e. cell density) scatter by FACScan.[23] We have analysed the cells present in this area (the 'monocyte area') which comprise approximately 20–30% of the total PBMC. Whether PBMC were fresh or cultured, most (> 75%) of the cells in the monocyte area expressed CD14 and CD33 antigens (see below). Since both markers are reportedly expressed by peripheral blood monocyte/macrophages, but not granulocytes, erythrocytes, lymphocytes, or platelets,[24] most of the cells in this area were considered to be monocytes.

To investigate the effect of $1,25\text{-}(OH)_2D_3$ on class II MHC expression in human monocytes, we analysed the cells in the monocyte area. Although most monocytes continued to express HLA-DR antigens after 3–5 days of culture (see Table 1), HLA-DR antigen expression was observed to be significantly increased on these cells (i.e. MFI of group A in Figs 1 and 2 was always greater than MFI on fresh cells, Table 1). This enhanced HLA-DR expression in culture (culture-enhanced HLA-DR) was found to be further augmented in the presence of IFN-γ (IFN-γ-enhanced HLA-DR, Fig. 1). Treatment with 100 nM $1,25\text{-}(OH)_2D_3$ for 4 days markedly reduced culture-enhanced HLA-DR as evidenced by MFI, i.e. the HLA-DR antigen density per cell, as well as the percentage of positive cells (MFI decreased from 601 to 137; percentage positive cells decreased from 90 to 55%). Moreover, $1,25\text{-}(OH)_2D_3$ significantly inhibited IFN-γ-enhanced HLA-DR to the level equivalent to that of untreated HLA-DR expression (Fig. 1, MFI, 1646 to 1637).

The inhibitory effect of $1,25\text{-}(OH)_2D_3$ on monocytes was found to be dose dependent and directed towards HLA-class II, but not class I expression (Table 1). Incubation of PBMC for 4 days with 1–100 nM $1,25\text{-}(OH)_2D_3$ reduced culture-enhanced

Table 1. 1,25-$(OH)_2D_3$ effectively inhibits class II but not class I HLA expression on PBMC

PBMC treatment*		MFI of cells in the monocyte area following incubation with:† GAM	Anti-HLA-class I	Anti-HLA-class II
Fresh		5	103 (>95)	57 (91)
Untreated:	Medium	16	157 (>95)	366 (90)
1,25-$(OH)_2D_3$	1 nM	10	130 (>95)	180 (89)
	10 nM	10	123 (>95)	106 (75)‡
	100 nM	12	121 (>95)	106 (55)‡
rIFN-γ	100 U/ml	12	171 (>95)	441 (92)
rIFN-γ	100 U/ml			
+ 1,25-$(OH)_2D_3$	100 nM	8	150 (>95)	288 (95)
Ethanol	0·1%	11	178 (>95)	374 (94)

* PBMC were cultured for 4 days in medium alone (untreated) or in the presence of 1,25-$(OH)_2D_3$ or rIFN-γ or both together.

† Cells were incubated with FITC-GAM alone, anti-HLA-ABC mAb followed by FITC-GAM, or with PE-conjugated anti-HLA-DR mAb (see Materials and Methods). Cells within the analytical gate (see Materials and Methods) were analysed for two-colour fluorescence by flow cytometry and the data are presented as mean fluorescence intensity, MFI and percentage positive (in parentheses).

‡ The percentage cells in 10 nM (75%) and 100 mM (55%) of 1,25-$(OH)_2D_3$ versus untreated (90%) is significantly different using the chi-square tests for independence.

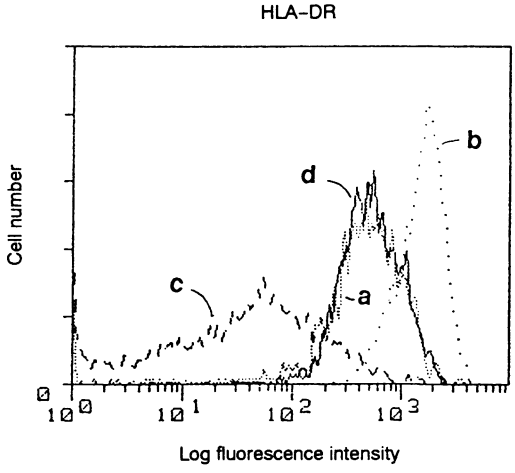

Figure 1. PBMC were cultured for 4 days in medium (15% FCS) (a), or in the presence of 100 U/ml IFN-γ (b), 100 nM 1,25-$(OH)_2D_3$ (c) or 100 U IFN-γ and 100 nM 1,25- $(OH)_2D_3$ (d). Cells in the monocyte area were then evaluated for HLA-DR expression (see Table 1 for details).

HLA-DR on cells in the monocyte area in a dose-dependent manner. Exposure to 0·1% ethanol [equivalent with that present during exposure to 100 nM 1,25-$(OH)_2D_3$] had no effect on HLA-DR expression, indicating that 1,25-$(OH)_2D_3$ was responsible for this inhibitory effect. Moreover, since there was only a marginal change in HLA-A, B and C expression, the inhibitory effect was apparently specific for HLA-DR (Table 1).

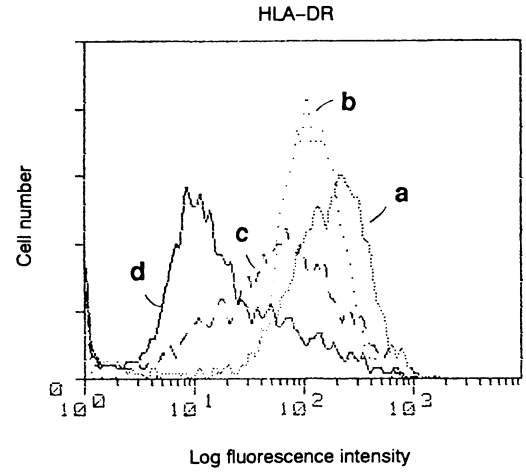

Figure 2. PBMC were cultured for 4 days in medium (15% FCS) (a) or in the presence of 1000 nM hydrocortisone (b), 1000 nM PGE_2 (c), or 100 nM 1,25-$(OH)_2D_3$ (d). The cells in the monocyte area were evaluated for HLA-DR expression. See Table 2 for further details.

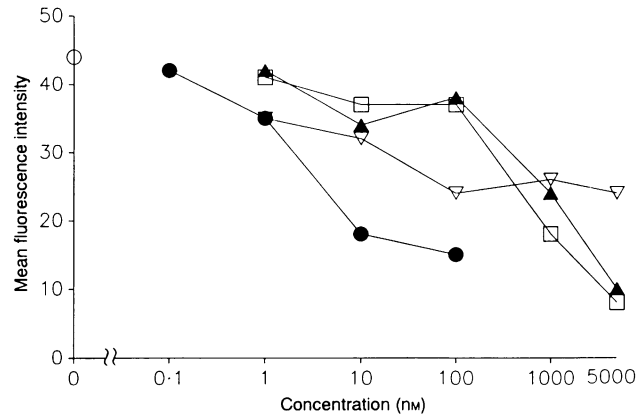

Figure 3. PBMC were cultured for 4 days in medium (15% FCS) (○) or varying concentrations of 1,25-$(OH)_2D_3$ (●), 24,25-$(OH)_2D_3$ (▲), 25-OHD_3 (□), or PGE_2 (▽). $CD33^+$ cells in the monocyte area were analysed for HLA-DR expression by using two-colour FACS analysis. Cultures containing ethanol concentrations equivalent to those in experimental samples had no discernible effect on HLA-DR expression (data not shown).

Effects of steroid hormones and PGE_2 on HLA-DR expression on peripheral blood monocytes

To compare the effect of D_3 with other biological molecules known to be capable of inhibiting class II in monocytes, PGE_2, and hydrocortisone, as well as T_3 (whose receptor shares most homology with the vitamin D_3 receptor) were examined. As shown in Fig. 2 and Table 2 (lines 1–5), at the concentration of 1000 nM (a concentration a log in excess of doses of PGE_2 which induced maximal inhibitory effect, see Fig. 3), these compounds also significantly inhibited culture-enhanced HLA-DR. However, their inhibitory effects did not appear to be as strong as that detected using 100 nM of 1,25-$(OH)_2D_3$ (76% reduction). Moreover, 100 nM of 1,25-$(OH)_2D_3$ again induced the strongest inhibition of IFN-γ-augmented HLA-DR expression compared with PGE_2 or T_3 (Table 2, lines 6–9).

Table 2. Comparison of HLA-DR inhibition on monocytes by 1,25-$(OH)_2D_3$ and other steroid hormones and PGE_2

PBMC treatment*		HLA-DR expression in monocyte area† % positive	MFI	% MFI‡
Untreated		92	210	100
PGE_2	1000 nM	88	107	51
Hydrocortisone	1000 nM	97	137	65
T_3	1000 nM	87	173	82
1,25-$(OH)_2D_3$	100 nM	68§	50	24
IFN-γ	100 U/ml	93	362	172
IFN-γ + PGE_2	100 U/ml 1000 nM	96	261	124
IFN-γ + T_3	100 U/ml 1000 nM	91	231	110
IFN-γ + 1,25-$(OH)_2D_3$	100 U/ml 100 nM	98	199	95

*PBMC were cultured for 4 days in medium alone (untreated) or in the presence of the indicated reagents.

† Cells were stained with PE-conjugated anti-HLA-DR mAb and the cells within the analytical gate were evaluated for per cent HLA-DR positive cells and MFI.

‡ The per cent MFI compares the MFI of the treated groups with the untreated (i.e. '100') cells.

§ The per cent positive cells incubated in 1,25-$(OH)_2D_3$ (68) versus untreated (92) is significantly different using the chi-square test for independence.

Inhibitory effect of 1,25-$(OH)_2D_3$ on HLA-DR expression of CD33$^+$ cells

Using two-colour FACS analysis, we examined the effect of 1,25-$(OH)_2D_3$ on HLA-DR expression on CD33$^+$ cells (Table 3). Incubation of PBMC for 4 days in culture resulted in up-regulation of HLA-DR on these cells compared with that on fresh or freshly frozen (usually slightly decreased MFI versus fresh cells) monocytes from this donor regardless of whether autologous or heterologous serum was used (group 1 versus 2 or 4). Treatment with 1,25-$(OH)_2D_3$ reduced culture-enhanced HLA-DR expression on the CD33$^+$ population (within the monocyte area) cultured in RPMI supplemented with FCS (15%) or autologous serum (15%), i.e. MFI group 2 versus 3, MFI 318 versus 107; group 4 versus 5, MFI 745 versus 240. In addition, 1,25-$(OH)_2D_3$ (100 nM) decreased IFN-γ-enhanced HLA-DR expression on CD33$^+$ cells below the level of untreated (group 7 versus 4, MFI 518 versus 745; untreated MFI 745). Although fresh frozen cells in the monocyte area almost all expressed CD33 antigens (95%), no large changes were observed in CD33 antigen expression itself in the total cultured cell population (i.e. approximately 80% of cultured cells expressed CD33).

Effect of vitamin D_3 analogues and PGE_2 on HLA-DR expression on CD33$^+$ cells

The vitamin D_3 analogues 24,25-$(OH)_2D_3$ and 25-$(OH)D_3$ have 500- to 1000-fold lower binding affinities for the vitamin D receptor (VDR) than 1,25-$(OH)_2D_3$. Although both compounds inhibited culture-enhanced HLA-DR on the CD33$^+$ population in a dose-dependent manner (Fig. 3), 100- to 1000-fold greater levels of both compounds compared with 1,25-$(OH)_2D_3$ were needed. Notably, their inhibitory effects on HLA-DR expression were profound but, even at high concentrations, not complete. PGE_2 was again observed to inhibit culture-enhanced HLA-DR expression in a dose-dependent manner but apparently not as effectively as 1,25-$(OH)_2D_3$. Indomethacin, which is capable of blocking PGE_2 production was utilized over a wide range of concentrations (0·01 nM–10 μM). However, indomethacin failed to block the down-regulation of MHC class II by 1,25-$(OH)_2D_3$ at all concentrations examined (data not shown).

Inhibitory effect of 1,25-$(OH)_2D_3$ on HLA-DR β-chain mRNA expression in monocyte-enriched adherent cells

To further ascertain the inhibitory effect of 1,25-$(OH)_2D_3$ on HLA-DR expression on monocytes, we examined mRNA expression of the HLA-DR β-chain in monocyte-enriched cell preparations. To enrich for monocytes, tightly adherent cells were obtained after overnight culture of PBMC and then treated with 1,25-$(OH)_2D_3$ alone, IFN-γ alone or both together for 48 hr. Total cellular RNA was prepared as described (see Materials and Methods). We carefully added equivalent amounts (10–20 μg) of each sample onto the gel in each experiment. As shown in the experiment in Fig. 4, HLA-DR β-chain-specific mRNA was clearly detected within the total mRNA preparations from monocyte-enriched cells cultured for 48 hr without treatment. Furthermore, IFN-γ treatment markedly up-regulated the expression of HLA-DR-specific mRNA (lane 3). Despite the possible activation of HLA-DR gene expression before treatment in these cell preparations because of prior overnight culture, 1,25-$(OH)_2D_3$ still clearly inhibited both culture-enhanced (35% suppression) and IFN-γ-enhanced (50% suppression) HLA-DR β-chain-specific mRNA expression as quantitated using a laser densitometer (Fig. 4).

DISCUSSION

We have recently reported that 1,25-$(OH)_2D_3$ can antagonize IFN-γ-induced expression of class II MHC antigens on rat thyroid follicular and mouse testicular Leydig cells.[17] These results were unexpected, since vitamin D_3 derivatives, alone or together with IFN-γ, have been reported to enhance the level of class II expression in a tumourigenic murine monocyte/macrophage cell line as well as a human promyelocyte population.[19,25] Therefore, the present studies were performed to determine if 1,25-$(OH)_2D_3$ would augment or diminish class II HLA-DR expression on normal human peripheral blood monocytes. We found that 1,25-$(OH)_2D_3$ profoundly inhibited both culture- and INF-γ-enhanced HLA-DR in a peripheral blood-cell population highly enriched for CD14 and CD33 antigen expression.

VDR appear to mediate most of the biochemical actions of vitamin D_3.[26] In the present study, the ability of several vitamin D_3 derivatives to diminish culture-enhanced HLA-DR expression was found to be dose dependent and directly proportional to their receptor-binding affinities. These results strongly suggested that the inhibitory effects observed require receptor–ligand interaction. Moreover, the inhibition of HLA-DR was

Table 3. Effect of 1,25-$(OH)_2D_3$ on HLA-DR expression of $CD33^+$ monocytes

Group	PBMC source*	Serum source	Treatment†	HLA class II expression (MFI) on: % $CD33^+$ cells‡	All cells‡	$CD33^+$ cells§
1	Cryopreserved		none	95	43	39
2	Fresh cultured	15% autologous	none	81	326	318
3	Fresh cultured	15% autologous	1,25-$(OH)_2D_3$	77	161	107
4	Fresh cultured	15% fetal calf	none	79	757	745
5	Fresh cultured	15% fetal calf	1,25-$(OH)_2D_3$	74	339	240
6	Fresh cultured	15% fetal calf	IFN-γ	79	928	944
7	Fresh cultured	15% fetal calf	1,25-$(OH)_2D_3$ + IFN-γ	85	539	518

* Freshly frozen (i.e. cryopreserved) cells which were rapidly thawed and not cultured or fresh cells cultured, i.e. 'fresh cultured' from the same individual, were evaluated for CD33 and HLA-DR expression by two-colour FACS analysis. Almost all of freshly frozen cells in the monocyte area expressed HLA-DR antigen (98%).

† PBMC were cultured for 4 days in medium containing 15% autologous serum or FCS alone or in the presence of 100 nM 1,25-$(OH)_2D_3$ or 100 U/ml rIFN-γ or both together.

‡ % $CD3^{3+}$ cells within the monocyte area.

§ MFI on all cells within the monocyte area and on $CD33^+$ cells within the monocyte area.

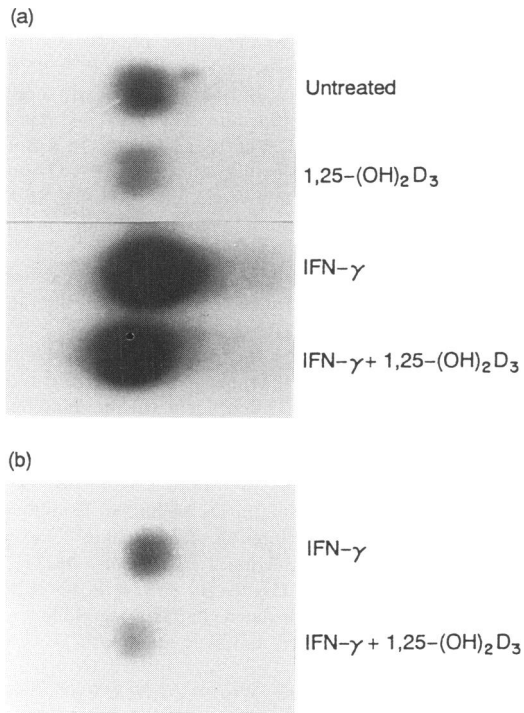

Figure 4. Monocyte-enriched (overnight adherence) PBMC were cultured for 2 days in medium (15% FCS) alone, in the presence of 100 nM 1,25-$(OH)_2D_3$ 100 U/ml IFN-γ, or 1,25-$(OH)_2D_3$ and IFN-γ. Total cellular RNA was prepared and 10 μg/lane was examined for the presence of HLA-DRβ mRNA. The hybridized filter was exposed to film for 20 hr (a) or 4 hr (b). The autoradiogram was scanned in a laser densitometer.

found to be dose dependent, and limited to class II, and not class I, HLA products. The inhibitory effect of 1,25-$(OH)_2D_3$ on both culture- and IFN-γ-enhanced monocyte HLA-DR was also detected at the mRNA level. Although steroid hormones in general, and specifically, analogues of 1,25-$(OH)_2D_3$ have been shown to alter the stability of specific mRNA,[27,28] further analyses are required in order to determine whether transcriptional and/or post-transcriptional effects are important in this inhibitory effect on HLA-DR antigen expression.

Although IFN-γ-induced class II expression on mouse Leydig cells was not inhibited by other steroid hormones and PGE_2, class II expression on monocyte/macrophages in either the absence or presence of IFN-γ has been reported to be down-regulated by such compounds.[7,9,10] We found that PGE_2, hydrocortisone and T_3 could down-regulate culture and IFN-γ- (data not shown) enhanced HLA-DR expression on human monocytes. However, in our studies, even at high concentrations they appeared to be less effective than 1,25-$(OH)_2D_3$. The explanation(s) for the difference in sensitivity to steroids and PGE_2 between the above-noted tissue cells and monocytes remains unknown. It may, however, be notable that class II MHC antigens are expressed constitutively in monocytes, but not in rat thyroid and mouse Leydig cells. Since monocytes treated with 1,25-$(OH)_2D_3$ may release increased amounts of PGE_2[29] products of the arachidonic acid pathway could be proposed to be responsible for mediating 1,25-$(OH)_2D_3$-induced inhibition. However, we did not find indomethacin capable of reversing the inhibitory activity of 1,25-$(OH)_2D_3$ (data not shown) suggesting that this effect is probably not mediated through D_3 induction of endogenous PGE_2.

In the present studies, greater than 0·1 nM of 1,25-$(OH)_2D_3$ had an inhibitory effect on culture-enhanced HLA-DR expression, indicating that 10-fold greater amounts versus normal serum 1,25-$(OH)_2D_3$ levels, (approximately 0·09 nM) were able to affect HLA-DR expression in monocytes.[30] Recently, significant amounts of 1-hydroxylase (1-OHase), which can convert 25-OHD_3 to the active 1,25-$(OH)_2D_3$ derivative, have been found to be produced in a variety of cell types and tissue other than kidney, including keratinocytes[31] and decidual tissue.[32] Notably, 1-OHase has also been detected in lymphocytes[33] and LPS- or IFN-γ-activated macrophages.[34] Thus, the local production and levels of 1,25-$(OH)_2D_3$ could be increased as a result of infiltrates containing activated lymphocytes and macro-

phages. Our finding would suggest that in such situations, the down-regulation of class II MHC expression in monocyte/macrophages as well as resident tissue cells could occur which might ultimately lead to diminution of antigen-presenting function by professional as well as non-professional (tissue cells induced to express class II) cell populations.

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