Regulation of Human Monocyte HLA-DR and CD4 Antigen Expression, and Antigen Presentation by 1,25-Dihydroxyvitamin D₃

By William F.C. Rigby, Mary Waugh, and Robert F. Graziano

1,25-Dihydroxyvitamin D₃ (1,25(OH)₂D₃) has been shown to be a macrophage-derived cytokine, capable of regulating myeloid differentiation and T-cell activation in vitro. Therefore, we examined the effects of 1,25(OH)₂D₃ on the monocyte phenotype and function of human peripheral blood monocytes as an index of its biologic role at an inflammatory site. 1,25(OH)₂D₃ treatment consistently and specifically reduced HLA-DR and CD4 expression by monocytes, while CD14 and class I HLA antigen expression were unaffected. Expression of FcγR I-III on monocytes was variably modulated by 1,25(OH)₂D₃ treatment, but no differences in antibody-dependent cell cytotoxicity (ADCC) were observed, measured using either ADCC or anti-FcγR-antibody expressing hybridomas. In contrast, the ability of monocytes to induce antigen-dependent T-cell proliferation was markedly reduced by 1,25(OH)₂D₃ pretreatment for as little as 6 hours. Addition of interleukin-1 (IL-1), IL-6, or indomethacin did not restore antigen-dependent T-cell proliferation, suggesting that this observation was not secondary to changes in IL-1, IL-6, or PGE₂ production induced by 1,25(OH)₂D₃. These data suggest that 1,25(OH)₂D₃ treatment specifically modulates human monocyte phenotype and function, altering HLA-DR antigen expression and antigen presentation, while leaving lytic function intact. These findings may be relevant to the immunobiologic role of 1,25(OH)₂D₃.

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Recognition of the wide distribution of specific receptors for vitamin D through all vertebrate cell types and tissues has prompted reassessment of the biologic role of this steroid hormone. Vitamin D compounds have been shown to exert potent growth inhibitory effects on a variety of tumor cell lines of hematopoietic or other origins. 1,25-Dihydroxyvitamin D₃ (1,25(OH)₂D₃), the most biologically active form of vitamin D, has shown to be an inducer of differentiation of both murine and human myeloid cell lines, resulting in decreased proliferative capacity, phenotypic monocytoid maturation, and enhanced effector cell function. Moreover, 1,25(OH)₂D₃ has been shown to be a potent immunoregulatory agent capable of modulating T-cell activation and lymphokine production.

These effects of 1,25(OH)₂D₃ on hematopoietic and lymphoid cells have assumed further relevance with the discovery that 1,25(OH)₂D₃ itself is a monocyte-macrophage-derived cytokine. Macrophage activation is accompanied by the acquisition of 1α-hydroxylase activity (1α-ΟHase) capable of converting 25-hydroxyvitamin D₃ (25-OHD) to 1,25(OH)₂D₃, the most biologically active form of vitamin D. Macrophage 1α-ΟHase appears to be comparable with its renal counterpart in substrate specificity, yet is distinctively regulated. It is inducible by γ interferon (IFN-γ) or lipopolysaccharide and is insensitive to parathyroid hormone. Production of 1,25(OH)₂D₃ by activated macrophages can be considerable, mediating systemic hypercalcemia in the absence of renal contribution. Reports of high levels of macrophage-derived 1,25(OH)₂D₃ have usually been associated with diseases of granulomatous histology.

Previous work has suggested that local production of 1,25(OH)₂D₃ by activated macrophages inhibits T-cell activation and IFN-γ synthesis, the stimulus that gives rise to its production. At an inflammatory site, monocytes migrating in from the blood would probably represent the least mature myeloid cells present. Therefore, we studied the effects of vitamin D compounds on monocyte phenotype and function as a model of their local role in myeloid differentiation at an inflammatory site. We report that vitamin D compounds specifically regulate class II HLA and CD4 antigen expression, as well as decrease the ability of monocytes to function as antigen-presenting cells. Thus, local production of 1,25(OH)₂D₃ by activated macrophages appears to regulate T-cell activation through accessory cell-dependent mechanisms. These findings further demonstrate a specific role for vitamin D compounds in modulating the immune response.

Materials and Methods

Reagents. Preservative-free 1,25-dihydroxycholecalciferol, 1,25(OH)₂D₃, and 25OH-D₃, the generous gifts of Drs Milan Uskokovic (Hoffman-La Roche Inc, Nutley, NJ) and Rodney Carlson (Upjohn Co, Kalamazoo, MI), were dissolved in 95% sterile ethanol, and stored in glass vials at −20°C. Human recombinant interleukin-1α (IL-1α) and IL-6 were generously provided by Dr Peter Lomedico (Hoffman-La Roche) and Genetics Institute (Cambridge, MA), respectively. Neutralizing IFN-γ antisera and a rabbit serum control obtained from the National Institutes of Health (NIH) Research Resources Section (Bethesda, MD) were used at a final concentration of 1:10: This reference antisera is capable of neutralizing 180 U IFN-γ/mL at this concentration. A neutralizing murine monoclonal antibody (MoAb), B3, generously provided by Dr Junming Le (New York University Medical School), was used at a final concentration of 1:10 and was capable of neutralizing 10 U IFN-γ/mL.

MoAbs. The following hybridomas were obtained from American Type Culture Collection (Rockville, MD) and their antibodies

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were always used under saturating conditions: W6/32 and L243, murine immunoglobulin Gα (IgGα) antibodies directed against monomorphic determinant of HLA A, B, and C and HLA-DR, respectively.2,22, OKT1, a rat IgGα-reactive antibody with CR3 (CD11b)24, HNK-1 (IgM), OKT4 (IgGα), OKT3 (IgGα), and OKT11 (IgGα) murine antibodies directed against NK cells, CD4, CD3, and CD2, respectively.23–25 AML-2-23 (IgGα) (anti-CD14), 32.2 (IgG) (anti-FcyRII), IV.3 (IgGα) (anti-FcyRIII), and 3G8 (anti-FcyRII) (anti-CD16) were generously provided by Dr Michael Fanger (Dartmouth Medical School), while Leu 11b (IgM) that binds CD16, OKDR (IgG) that binds HLA-DR, and BA-1 (IgM) that binds CD24, were purchased as ascites from Becton-Dickinson (Mountain View, CA), Ortho Diagnostic Systems (Raritan, NJ), and Boehringer Mannheim (Indianapolis, IN).26–29

Cell purification. Monocytes were obtained by cytophoresis of normal volunteers. Cells from cytophoresis packs were spun on Ficoll-Hypaque and the interface layer collected. After two washes in RPMI, the cells were resuspended in RPMI supplemented with 10% heat-inactivated fetal bovine serum (FBS) at 5 × 10^6/mL in 15-mL polypropylene tubes and rotated at 8 rpm for 1 hour at 4°C to induce monocyte clumping as previously described.26 Clumping was not found to alter monocyte phenotype or cytotoxic effector function. The cell suspension (2 mL) was then layered onto an equal volume of nuclease-free ammonium bromide-treated sheep erythrocytes, resulting in T-cell purity greater than 98%.27 The percentage of cells expressing CD14 (as determined by AML-2-23 binding) was usually equivalent or slightly less (less than 5%) than the percentage of cells that were nonspecific esterase-negative. Purified T lymphocytes were prepared from nonclumping cells by plastic adherence, followed by rosetting with aminophenylisothiocyanate bromide-treated sheep erythrocytes, resulting in T-cell purity consistently greater than 90% as determined by OKT3 binding. T-lymphocyte preparations were further depleted of accessory cells by treatment at 4°C with L243 and AML-2-23, followed by two cycles of complement treatment and three washes. As a consequence of these treatments, no AML-2-23 or L243-positive cells were detectable by flow cytometry. T cells were then incubated at 5 × 10^6 cells/mL in RPMI 1640 supplemented with 10% autologous sera in tissue culture flasks (Becton Dickinson, Oxnard, CA) at 37°C in a 5% CO₂, humidified atmosphere until needed.

Cell cultures. Monocytes were cultured at 2 × 10^6/mL in Teflon vessels (Savillex, Minnetonka, MN) at 37°C in 5% CO₂, humidified atmosphere, in RPMI 1640 supplemented with either autologous or heat-inactivated FBS (Hazelton Research Products, Denver, PA) and 20 μg/mL gentamicin sulfate (US Biochemical, Cleveland, OH). Comparison cultures were performed using both autologous and FBS in both the absence and presence of 10-mm HEPES and 5 × 10⁻² mol/L β mercaptoethanol (Sigma Chemical Co, St Louis, MO). Monocytes were cultured with vitamin D metabolites or an ethanol control, with the final ethanol concentration never exceeding 0.01%. No significant differences in vitamin D activity were observed as measured by changes in cell phenotype under any of these conditions. Furthermore, comparable vitamin D activity on monocyte differentiation was observed using both unpurified and purified monocytes. Thus, the effects of vitamin D metabolites on monocyte phenotype appeared to be consistent despite varied culture conditions and monocyte purity. Furthermore, cell viability was comparable between control and 1,25(OH)₂D₃-treated cultures under each set of conditions.

Cytofluorograph analysis. Monocytes or lymphocytes prepared as described were washed in phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) and 0.05% sodium azide. One million pelleted cells were mixed with 25 μL of purified human IgG1 (10 mg/mL) and saturating concentrations of the relevant MoAb. After a 1-hour incubation on ice, cells were washed once with PBS-BSA-sodium azide, and treated for 45 minutes on ice with 25 μL of a saturating concentration (1:20 dilution) of FITC-conjugated affinity-purified F(ab)′; goat anti-mouse Ig (FITC-GAM) (Boehringer-Mannheim). Cells were washed, the pellet disrupted, and the cells fixed with 250 μL of ice-cold 2% paraformaldehyde in PBS. The Ortho (Westwood, MA) Cytofluorograph System 50H was used to quantify binding of MoAb. Similar results were obtained using fixed or fresh cells. Nonspecific binding obtained using irrelevant IgG or IgM MoAbs is subtracted to yield specific fluorescence. All samples were run in duplicate.

To more accurately quantify cell-associated immunofluorescence between experiments, the instrument was calibrated before assay with quantitative fluorescein micro bead standards.28 (Flow Cytometry Standards Corp, Research Triangle Park, NC). A standard curve was constructed by plotting FITC molecules per bead versus log of mean fluorescence intensity. In the experiments, samples were run in duplicate, and the mean and SD were calculated. Relative MoAb sites per cell, which represents the binding of FITC-GAM per cell, were calculated by dividing the number of FITC molecules bound per cell by the fluorescein to protein ratio of the FITC-GAM. Unless otherwise indicated, relative MoAb sites per cell represents specific binding of FITC-GAM (ie, the binding after subtracting the value of the irrelevant isotype control MoAb). Statistical analysis was performed using independent t-tests where raw data was analyzed with the MYSTAT computer software program (SYSTAT, Evanston, IL).

Cytotoxicity assays. To quantify direct cytotoxicity of hybridoma cells, equal volumes of medium, [³¹Cr]-labeled hybridoma target cells, and effector cells were mixed in round-bottom microtiter wells as previously described.29 Plates were incubated for 6 hours at 37°C, after which half of the supernatant was removed and counted for release of [³¹Cr]. Maximal lysis was obtained by addition of 2% sodium dodecyl sulfate in water. Percentage of cytotoxicity was calculated as 100 × (counts released with effectors – spontaneous lysis) / (maximum lysis – spontaneous lysis).

Antibody-dependent cell cytotoxicity (ADCC) was performed as previously described.30 Briefly, freshly drawn chicken erythrocytes (CE) were washed in RPMI 1640, incubated with 200 μCi of [³¹Cr]-sodium chromate (New England Nuclear, Boston, MA) for 1 ½ hours at 37°C, washed, and resuspended at 2 × 10⁶ cells/mL in medium 199 containing 20% FBS. Equal volumes (50 μL) of labeled CE targets, either control or treated effector cells, and anti-CE IgG at the concentrations indicated were mixed in round-bottomed microtiter wells (A/S Nunc, Denmark) and incubated for 4 hours, after which half the supernatant was removed for determination of [³¹Cr] release. Maximal lysis was achieved by addition of 100 μL of 2% detergent (“Count Off,” New England Nuclear). The percentage of cytotoxicity was determined according to the equation: % cytotoxicity = ([release of Ab] – [release without Ab])/([maximum lysis – spontaneous release].

Antigen presentation. In these experiments, monocytes were cultured in RPMI 1640 with 10% autologous serum as described in the relevant figure or table, washed three times in RPMI 1640, and resuspended in RPMI-10% autologous sera for 2 hours at 37°C.
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irradiated (5,000 R), and washed again. Monocytes (10⁷/mL) were added to washed, purified T cells (10⁶/mL final) in the absence or presence of preservative-free tetanus toxoid and cultured for the specified times. The percentage of monocytes used was selected as it induced optimal antigen-dependent proliferation of T cells at 5 and 7 days. IL-1 and IL-6 were used at concentrations that provided optimal comitogenic activity with antigen- and phytohemagglutinin-induced proliferation (0.5 µg/mL) (Wellcome, Beckenham, England).

Proliferation studies. After various times in culture, equal volumes (100 µL) of each culture (T cells ± either irradiated control or treated monocytes ± tetanus toxoid) were plated in triplicate into 96 well plates (Becton Dickinson Labware, Oxnard, CA). Cells were pulsed with 1.0 µCi of [³H]-Tdr (70 Ci/mmol, New England Nuclear) overnight at 37°C, and harvested onto glass fiber filters and counted by liquid scintillation.

RESULTS

Regulation of monocyte phenotype by 1,25(OH)₂-D. Monocytes cultured for 40 hours in the presence of 10 nmol/L 1,25(OH)₂-D were analyzed by indirect immunofluorescence for phenotypic changes using a panel of MoAbs. We found that 1,25(OH)₂-D treatment consistently reduced the expression of HLA-DR and CD4 antigens (Fig 1). Expression of FcyRI detected by the 32.2 was variably affected by 1,25(OH)₂-D treatment (vide infra), while AMI-2-23, OKM1 (directed against CR3), IV.3, 3G8, and W6/32 (directed against HLA-A, B, C) binding were unaffected (data not shown).

In ensuing work, fluorescent bead standards were used to enable direct comparison between experiments. Time-course experiments showed that the 1,25(OH)₂-D-mediated decrease in HLA-DR antigen expression evident at 40 hours was also apparent at 3 and 7 days of culture (P < .05), as were changes in 32.2 and OKT4 binding (Fig 2). No significant decrease in HLA-DR or CD4 expression was observed before 16 hours of culture with 1,25(OH)₂-D (data not shown). The reduction in HLA-DR and CD4 antigen expression was concentration-dependent, first evident at 1 nmol/L and becoming maximal at 10 nmol/L. 25(OH)-D (Fig 3) had at least 10-fold less activity in reducing HLA-DR expression, while having no effect on CD4 expression, thereby suggesting the specificity of these effects. Interestingly, in this donor neither vitamin D metabolite affected FcyRI expression as detected by 32.2 binding. The variability of the effect of vitamin D compounds on FcyRI expression by monocytes was not serum-dependent, as 1,25(OH)₂-D treatment-mediated comparable changes in 32.2, OKT4, and OKDR binding when cultures were performed in either autologous or fetal calf serum (data not shown). Moreover, 1,25(OH)₂-D (10 nmol/L) mediated similar decreases in HLA-DR expression in monocytes cultured in autologous or nonautologous human serum (39% and 41% of control, respectively). In other experiments (data not shown), the effects of 1,25(OH)₂-D on monocyte class II and CD4 antigen expression have been shown to be independent of medium, supplements (HEPES buffer and β-mercaptoethanol), and contaminating lymphocytes, suggesting that these effects are mediated solely and directly by 1,25(OH)₂-D on the monocyte.

For two reasons, it seemed unlikely that any observed effects of 1,25(OH)₂-D on monocyte phenotype were secondary to inhibition of IFN-γ produced by contaminating lymphocytes. First, CD3⁺ cells were undetectable by flow cytometry. Second, IFN-γ has been reported to decrease monocytic CD4 expression and increase HLA-DR expression. If 1,25(OH)₂-D-mediated effects on these antigens' expression were IFN-γ-dependent, CD4 expression should increase, not decrease in concert with HLA-DR antigen expression. To resolve this more completely, we examined the ability of polyclonal and MoAbs to IFN-γ to abrogate the effects of 1,25(OH)₂-D on HLA-DR and CD4.
antigen expression (Table 1). Addition of anti-IFN-γ antibody or an isotype control antibody were compared for their effects on monocytes cultured with 1,25(OH)2-D. Anti-IFN-γ antibody did not substantially affect HLA-DR expression by either control or 1,25(OH)2-D–treated monocytes in either experiment, further indicating that this effect of 1,25(OH)2-D on monocyte phenotype was mediated directly. The reduction of HLA-DR expression by 1,25(OH)2-D in the presence of anti-IFN-γ antibody was significant (P = .004). In contrast, a small increase in CD4 expression was seen with anti-IFN-γ antibody in 1,25(OH)2-D–treated cells. Because no corresponding increase in CD4 expression was seen in control cultures, this may represent a nonspecific effect of the rabbit antiserum.

Regulation of ADCC by 1,25(OH)2-D. Work in our laboratory had shown that ADCC by myeloid cell lines was enhanced by treatment with 1,25(OH)2-D, even in the presence of decreased numbers of FcγR. These data, as well as the report by Cohen et al demonstrating increased oxygen radical formation by 1,25(OH)2-D–treated monocytes, suggested a potential role in monocyte ADCC. We were unable to demonstrate any change in ADCC in monocytes treated with 1,25(OH)2-D compared with controls at an effectortarget ratio of 20:1 (ETOH: 32.8% ± 2.0%; 1,25(OH)2-D: 36.8% ± 5.0%, mean ± SEM, four experiments). Similar lack of effect was observed at effector:target ratios of 100:1 and 4:1. This was paralleled by comparable levels of FcγR types I-III expression between control and treated monocytes. Cultured monocytes express three different FcγR, all capable of mediating ADCC. Using hybridomas expressing high surface levels of antibodies directed against each FcγR as targets, we examined the ability of 1,25(OH)2-D to selectively modulate cytotoxic function by these different FcγR. Cytotoxic activity transduced by all three FcγR was unaffected by 1,25(OH)2-D. These data suggest that, unlike myeloid cell lines, the lytic capability of the monocyte was not significantly altered by short-term 1,25(OH)2-D treatment. Finally, we observed little or no natural killing of K562 erythroleukemia cells (an index of natural killer activity) at effector:target ratios of 100:1 in our monocyte preparations, and no effect of
**Table 1. Effect of Anti-IFN-γ Antibody on Phenotypic Activity of 1,25(OH)₂-D**

<table>
<thead>
<tr>
<th>Anti-IFN-γ</th>
<th>1,25(OH)₂-D</th>
<th>L243 (antibody molecules/cell x 10⁻⁵)</th>
<th>OKT4</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−</td>
<td>−</td>
<td>36.4 ± 3.2</td>
<td>12.8 ± 0.8</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>15.3 ± 0.4</td>
<td>7.4 ± 0.2</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>29.0 ± 1.6</td>
<td>12.0 ± 2.2</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>18.1 ± 0.4</td>
<td>9.1 ± 0.4</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>26.4 ± 0.3</td>
<td>ND</td>
</tr>
<tr>
<td>−</td>
<td>+</td>
<td>15.5 ± 0.7</td>
<td>ND</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
<td>25.9 ± 0.9</td>
<td>ND</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>15.7 ± 1.6</td>
<td>ND</td>
</tr>
</tbody>
</table>

Monocytes were isolated and cultured for 40 hours as described in Materials and Methods in experiment 2. Monocytes were purified by cold aggregation only. Rabbit-derived anti-IFN-γ antisera with normal rabbit sera as a control was used in experiment 1, while the murine anti-IFN-γ MoAb B3 was used in experiment 2. Flow cytometric analysis was performed after 40 hours.

Abbreviation: ND, not done.

1,25(OH)₂-D treatment. This lack of antibody-independent cytotoxic activity also indicates that contamination of our monocyte preparations by NK cells was functionally undetectable.

**Regulation of monocyte antigen presentation by 1,25(OH)₂-D.** Due to the critical role of class II major histocompatibility complex molecules in antigen presentation, we examined whether pretreatment of purified monocytes with 1,25(OH)₂-D, followed by extensive washing, would affect their ability to present antigen to purified autologous T cells (Table 2). Using a percentage of monocytes (10%) that gave optimal stimulation, pretreatment (16 to 40 hours) of monocytes with various concentrations of 1,25(OH)₂-D resulted in a dose-dependent decrease in their ability to induce T-cell proliferation in the presence of tetanus toxoid. These effects became maximal with 1 to 10 nmol/L 1,25(OH)₂-D. Pretreatment of monocytes with 25OH-D at equivalent concentrations augmented proliferation over that seen with an ethanol control, demonstrating the specificity of this inhibitory activity of 1,25(OH)₂-D (experiment 3). Moreover, these effects were antigen-dependent, as in the absence of tetanus toxoid no significant [³H]-TdR incorporation was observed. Comparable dose-responsiveness was seen with peripheral blood mononuclear cells as well as fractionated cells, indicating that fractionation had not altered cell sensitivity to 1,25(OH)₂-D (data not shown).

To determine if an association of 1,25(OH)₂-D-mediated changes in HLA-DR expression with altered monocyte antigen presenting was present, we correlated the rates with which these two parameters were modulated (Fig 4). Short-term (less than 6 hours) exposure of monocytes to 1,25(OH)₂-D markedly impaired antigen-presenting capacity (APC) by monocytes. The rate of the effects of 1,25(OH)₂-D on APC emerged much earlier than those involving surface HLA-DR antigen expression. However, the degree of decrease in APC and HLA-DR expression did seem to correlate within, as well as between, donors after 40 hours of treatment. Finally, examination of proliferation at multiple time points (3 to 7 days) suggested that these differences were not due to altered kinetics of antigen-dependent T-cell activation.

In other experiments, we examined the ability of monocyte-derived comitogens (IL-1, IL-6) to reverse the effects of 1,25(OH)₂-D on APC. Addition of recombinant IL-1 (5 to 20 U/mL) to cultures augmented antigen-induced T-cell proliferation induced by control and 1,25(OH)₂-D–treated monocytes, but did not overcome the marked differential in their respective ability to induce T-cell proliferation (Table 3). Combinations of optimal concentrations of IL-1 and IL-6 did not reverse this defect in monocyte APC either. Higher concentrations of IL-1 (experiment 3) as well as IL-6 (data

**Table 2. Effect of Various Concentrations of Vitamin D Compounds on Antigen Presentation by Monocytes**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tetanus Toxoid</th>
<th>Exp 1</th>
<th>Exp 2</th>
<th>Exp 3</th>
<th>Exp 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>+</td>
<td>6.2 ± 0.2</td>
<td>11.0 ± 0.2</td>
<td>11.2 ± 0.8</td>
<td>5.5 ± 0.2</td>
</tr>
<tr>
<td>1,25(OH)₂-D (10⁻¹ mol/L)</td>
<td>+</td>
<td>ND</td>
<td>9.9 ± 0.5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>1,25(OH)₂-D (10⁻¹⁰ mol/L)</td>
<td>+</td>
<td>4.9 ± 0.2⁰</td>
<td>5.6 ± 0.1⁰</td>
<td>12.7 ± 1.3</td>
<td>4.2 ± 0.1⁰</td>
</tr>
<tr>
<td>1,25(OH)₂-D (10⁻² mol/L)</td>
<td>+</td>
<td>2.5 ± 0.3⁰</td>
<td>5.1 ± 0.1⁰</td>
<td>9.1 ± 0.5†</td>
<td>3.1 ± 0.4⁰</td>
</tr>
<tr>
<td>1,25(OH)₂-D (10⁻⁸ mol/L)</td>
<td>+</td>
<td>1.8 ± 0.1⁰</td>
<td>7.2 ± 0.5⁰</td>
<td>7.3 ± 0.2⁰</td>
<td>2.2 ± 0.1⁰</td>
</tr>
<tr>
<td>1,25(OH)₂-D (10⁻¹⁰ mol/L)</td>
<td>+</td>
<td>1.9 ± 0.2⁰</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>25OH-D (10⁻⁹ mol/L)</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>19.8 ± 1.3</td>
<td>ND</td>
</tr>
<tr>
<td>25OH-D (10⁻⁸ mol/L)</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>17.1 ± 0.2</td>
<td>ND</td>
</tr>
<tr>
<td>Control</td>
<td>−</td>
<td>ND</td>
<td>0.2 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>1,25(OH)₂-D (10⁻¹⁰ mol/L)</td>
<td>−</td>
<td>ND</td>
<td>0.3 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.3 ± 0.1</td>
</tr>
</tbody>
</table>

Monocytes were isolated and cultured as described in Materials and Methods with an ethanol control or various concentrations of 1,25(OH)₂-D or 25OH-D compounds for 16 (experiments 1 and 2) or 40 hours (experiment 3), washed extensively, and irradiated. Monocytes (10⁶/mL final concentration) were then added to purified T cells (10⁵/mL) in the absence or presence of tetanus toxoid (1 μg/mL) cultured for 5 days and then pulsed for 24 hours with [³H]-TdR. Data shown represent the mean and SD of samples performed in triplicate.

Abbreviation: ND, not done.

*P ≤ 0.001.
†P ≤ 0.02.
cytometry for specific antibody molecules bound/cell at the time cultured Xor various times with ethanol or 1,25(OH),-D (10 nmol/L). Moreover, the inclusion of their coculture with T cells and tetanus toxoid.

The discovery that activated macrophages synthesize 1,25(OH),-D under the regulation of IFN-γ has resulted in multiple studies attempting to discern its biologic role in terms of the immune response. In this report, we show that 1,25(OH),-D treatment of human monocytes consistently and specifically reduces their expression of HLA-DR and CD4 antigens. Studies of the functional consequences of these phenotypic changes showed that 1,25(OH),-D had little or no effect on ADCC by monocytes. In contrast, pretreatment of monocytes with 1,25(OH),-D markedly impaired their ability to induce antigen-dependent proliferation of purified T lymphocytes. Thus, exposure of monocytes to 1,25(OH),-D selectively modulates monocyte differentiation and function, resulting in changes in monocyte–T-cell interactions, but not lytic effector capability.

Previous work has demonstrated that vitamin D compounds inhibit T-cell activation and proliferation in both human and murine systems. In this report, we show that monocyte pretreatment with 1,25(OH),-D alters the ability of monocytes to activate T cells in an antigen-dependent manner. 1,25(OH),-D treatment of monocytes has been reported to augment their production of PGE2, a potent inhibitor of T-cell proliferation. Therefore, it was possible that the reduction in T-cell proliferation seen in the presence of 1,25(OH),-D–treated monocytes was secondary to increased secretion of PGE2. Several lines of evidence suggest that a PGE2-dependent mechanism could not account for these effects of 1,25(OH),-D on monocyte function. First, addition of indomethacin to cultures had little or no effect on the decreased T-cell proliferation seen with 1,25(OH),-D

Table 3. Effect of IL-1 and IL-6 on Antigen Presentation by 1,25(OH),2-D–Treated Monocytes

<table>
<thead>
<tr>
<th>Tetanus Toxoid</th>
<th>1,25(OH),2-D</th>
<th>[3H]-Tdr (cpm x 10^-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T cells + monos</td>
<td>+</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10.0 ± 0.2</td>
</tr>
<tr>
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<td>+</td>
<td>56.3 ± 9.0</td>
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<td>6.2 ± 0.2</td>
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<td>24.8 ± 0.9</td>
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<tr>
<td>T cells + monos + IL-1</td>
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<td>0.2 ± 0.1</td>
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<tr>
<td>T cells + monos + IL-1/IL-6</td>
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<td>4.4 ± 0.3</td>
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<td>0.6 ± 0.1</td>
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<td>3.7 ± 0.3</td>
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<tr>
<td>T cells + monos</td>
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<td>57.1 ± 1.1</td>
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<td>46.7 ± 1.0</td>
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<td>+</td>
<td>7.5 ± 0.5</td>
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Abbreviation: ND, not done.
pretreatment of monocytes. Second, addition of untreated monocytes to 1,25(OH)\(_2\)-D-treated monocytes restored antigen-induced proliferation of T cells to control levels, suggesting that these changes were not secondary to increased production of PGE\(_2\), other growth inhibitory factors, or the consequence of residual (despite extensive washing) 1,25(OH)\(_2\)-D. Moreover, the decrease in antigen-specific T-cell proliferation seen with 1,25(OH)\(_2\)-D pretreatment of monocytes was unaffected by exogenous IL-1 alone or in combination with IL-6, suggesting that this observation was not due to decreased production of these soluble factors. The IL-1 independence of these effects of 1,25(OH)\(_2\)-D is consistent with the report that 1,25(OH)\(_2\)-D increases IL-1 production by human monocytes.\(^{49}\)

Therefore, these data would suggest that 1,25(OH)\(_2\)-D mediates these effects through alterations in the ability of monocytes to present antigen. Given the role of HLA class II antigens in antigen recognition by the T cell,\(^{23,43}\) a decrease in the density of HLA-DR antigens on the surface of a monocyte might result in a significant reduction in its APC.

Reduction in the total level of cell surface expression of HLA-D region products might effect a critical decrease in the density of HLA-DR antigen complexed with the processed foreign antigen, resulting in decreased T-cell recognition and triggering of the CD3-antigen receptor complex. A direct correlation between the level of I\(\alpha\) expression and APC has been reported with murine macrophages,\(^{46}\) which we also observed in our examination of the concentration-dependence of this effect of 1,25(OH)\(_2\)-D.

In the kinetic experiments, the failure to observe correlation between the rates at which APC and surface HLA-DR expression are modulated is not surprising, since antigen primarily associates with newly synthesized class II major histocompatibility gene products.\(^{45}\) Thus, if 1,25(OH)\(_2\)-D treatment inhibits HLA-DR biosynthesis, decreased complexing of antigen with HLA-DR would be expected, resulting in decreased antigen-induced proliferation. If the rate of turnover of plasma membrane HLA-DR antigen does not occur rapidly, reduced levels of antigen complexed to newly synthesized HLA-DR (and reduced antigen-induced proliferation) would be apparent before any observable changes in the total level of HLA-DR expression on the cell surface. To examine this hypothesis, we are attempting to determine both the mechanisms and kinetics by which 1,25(OH)\(_2\)-D regulates HLA-DR antigen expression. Alternatively, 1,25(OH)\(_2\)-D treatment may affect uptake and processing of antigen alone or in concert with the reduction in HLA-DR antigen expression. An additional potential mechanism is that 1,25(OH)\(_2\)-D treatment might decrease expression of adhesion molecules (LFA-3, ICAM-1) that have been shown to be important in monocyte-T-cell interactions.\(^{46}\) Preliminary data suggest that LFA-3 and ICAM-1 expression on monocytes is unaffected by 1,25(OH)\(_2\)-D treatment (Rigby WFC, Waugh MG, manuscript submitted). Each of these possibilities might, in conjunction with decreased HLA-DR expression, contribute to the decrease in APC by 1,25(OH)\(_2\)-D-treated monocytes, and are currently under study.

It could be argued that these effects of 1,25(OH)\(_2\)-D on APC are being mediated not through the monocyte but through another cell capable of presenting antigen (B lymphocytes or dendritic cells), persisting despite extensive monocyte purification (95% CD14\(^+\) in some preparations). B lymphocytes seem an unlikely candidate for this activity for several reasons, among them their failure to express receptors for vitamin D unless activated,\(^{46}\) the consistent presence of less than 5% B cells in our preparations, and the ability of irradiation (used in our protocols) to ablate B-cell APC.\(^{44}\) Dendritic cells, a specialized antigen-presenting cell present in the peripheral blood in very low levels (0.1% to 0.5%), cannot be conclusively proven or disproven to be present in our preparations, as they lack any specific cell-associated antigens and CD14.\(^{45}\) If these effects of 1,25(OH)\(_2\)-D on antigen presentation are mediated through its effects on dendritic cells, one would have to postulate this occurrence despite a tremendous excess of monocytes, as well as assume that peripheral blood dendritic cells express vitamin D receptors.

In conclusion, these studies suggest that 1,25(OH)\(_2\)-D selectively modulates the expression of CD4 and HLA-DR...
antigen expression by human monocytes. Perhaps solely as a consequence of these effects on surface phenotype, 1,25(OH)$_2$-D inhibits the ability of purified monocytes to present antigen through an IL-1 and IL-6-independent mechanism. ADCC was unaffected by 1,25(OH)$_2$-D treatment. Other studies have shown that anti-mycobacterial lysozyme secretion, tumor necrosis factor production, and heat shock protein synthesis by human peripheral blood monocytes are augmented by 1,25(OH)$_2$-D. Local production of the monokine, mononuclear lymphocytes, augments through an association with a factor from human T lymphocytes, augments of 25-hydroxyvitamin D, by cultured pulmonary alveolar macrophages may therefore influence monocytes migrating into an inflammatory site to adopt a specific functional agenda. Our findings would suggest that 1,25(OH)$_2$-D may limit the immune response in granulomata through decreased antigen presentation mediated both directly on the monocytes as well as indirectly through inhibition of IFN-γ production. Moreover, similar effects of 1,25(OH)$_2$-D on HLA-D region product expression have been reported with human melanoma cells, suggesting that this effect of 1,25(OH)$_2$-D may be common to other cells capable of class II HLA antigen expression. This finding, as well as our own, would suggest that 1,25(OH)$_2$-D may function as a cytokine that specifically modulates the expression of HLA-DR expression by potential antigen presenting cells. In conclusion, based on our studies, 1,25(OH)$_2$-D exerts discrete effects on monocyte phenotype and function, with particular pertinence to monocyte–T-cell interactions at an inflammatory site. This selective activity of 1,25(OH)$_2$-D may be responsible for its observed synergy with cyclosporin A in inhibiting T-cell activation. Thus, 1,25(OH)$_2$-D may not only represent an important cytokine in the formation and perhaps eventual resolution of granulomata, but may also have pharmacologic application in immunosuppressive therapy.

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Regulation of human monocyte HLA-DR and CD4 antigen expression, and antigen presentation by 1,25-dihydroxyvitamin D3

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