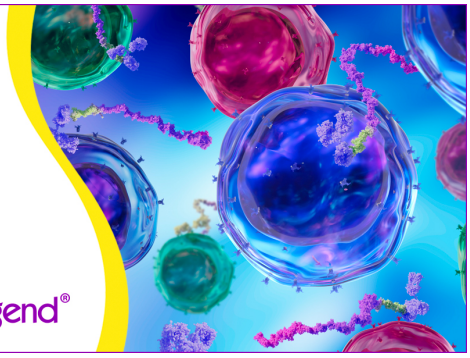
TotalSeq™ PhenoCyte
Ultra-high parameter, high-throughput, single-cell protein profiling
Learn more
BioLegend®


The Journal of Immunology
RESEARCH ARTICLE | MAY 01 2000

Vitamin D_3 Affects Differentiation, Maturation, and Function of Human Monocyte-Derived Dendritic Cells[1] FREE

Lorenzo Piemonti; ... et. al

J Immunol (2000) 164 (9): 4443–4451.
https://doi.org/10.4049/jimmunol.164.9.4443

Related Content

A Vitamin D Analog Down-Regulates Proinflammatory Chemokine Production by Pancreatic Islets Inhibiting T Cell Recruitment and Type 1 Diabetes Development

J Immunol (August,2004)

Transcriptional Profiling of γδ T Cells Identifies a Role for Vitamin D in the Immunoregulation of the Vγ9Vδ2 Response to Phosphate-Containing Ligands

J Immunol (May,2005)

Developmental Vitamin D Deficiency and the Vitamin D Receptor Control Hematopoiesis

J Immunol (September,2024)

Downloaded from http://journals.aai.org/jimmunol/article-pdf/164/9/4443/1118540/4443.pdf by guest on 14 May 2025

Vitamin D_3 Affects Differentiation, Maturation, and Function of Human Monocyte-Derived Dendritic Cells[1]

Lorenzo Piemonti,[2]*‡ Paolo Monti,‡ Marina Sironi,§ Paolo Fraticelli,§ Biagio Eugenio Leone,†‡ Elena Dal Cin,† Paola Allavena,§ and Valerio Di Carlo*‡

We studied the effects of 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$) on differentiation, maturation, and functions of dendritic cells (DC) differentiated from human monocytes in vitro in the presence of GM-CSF and IL-4 for 7 days. Recovery and morphology were not affected by 1α,25-$(OH)_2D_3$ up to 100 nM. DC differentiated in the presence of 10 nM 1α,25-$(OH)_2D_3$ (D_3-DC) showed a marked decrease in the expression of CD1a, while CD14 remained elevated. Mannose receptor and CD32 were significantly increased, and this correlated with an enhancement of endocytic activity. Costimulatory molecules such as CD40 and CD86 were slightly decreased or nonsignificantly affected (CD80 and MHC II). However, after induction of DC maturation with LPS or incubation with CD40 ligand-transfected cells, D_3-DC showed marginal increases in MHC I, MHC II, CD80, CD86, CD40, and CD83. The accessory cell function of D_3-DC in classical MLR was also inhibited. Moreover, allogeneic T cells stimulated with D_3-DC were poor responders in a second MLR to untreated DC from the same or an unrelated donor, thus indicating the onset of a nonspecific hyporesponsivity. In conclusion, our data suggest that 1α,25-$(OH)_2D_3$ may modulate the immune system, acting at the very first step of the immune response through the inhibition of DC differentiation and maturation into potent APC. ***The Journal of Immunology,*** **2000, 164: 4443–4451.**

1α,25-Dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$)[3] is a secosteroid hormone that binds to a nuclear receptor named vitamin D_3 receptor. During the past few years it has become apparent that 1α,25-$(OH)_2D_3$, in addition to its well-known role in mineral and skeletal homeostasis, regulates the differentiation, growth, and function of a broad range of cells, including cells of the immune system (1–4). The immunological effects of pharmacological levels of 1α,25-$(OH)_2D_3$ or its analogues in vivo were demonstrated in studies of autoimmune disease (5–16) and studies of allograft rejection (17–20). The effects of 1α,25-$(OH)_2D_3$ on the immune system were ascribed to its action on lymphocytes and monocytes/macrophages (21–23). When added to mitogen-stimulated human peripheral blood lymphocytes in vitro, 1α,25-$(OH)_2D_3$ inhibits their proliferation, Ig synthesis, and accumulation of transcripts for IL-1, IL-2, IL-6, TNF-α, and -β and IFN-γ (24–26). Of interest is that 1α,25-$(OH)_2D_3$ induces promyelocytes to differentiate into monocytes (27); in addition, 1α,25-$(OH)_2D_3$ differentiates myeloid leukemia cells to nonproliferating monocyte/macrophage-like cells in both humans and mice (28, 29) and promotes the differentiation of myeloid stem cells and normal peripheral blood monocytes toward a macrophage phenotype (30). 1α,25-$(OH)_2D_3$ also affects functional activities of monocytes and macrophages with contrasting results. Tumor cell cytotoxicity, phagocytosis, and mycobactericidal activity of monocytes/macrophages are enhanced by exposure to 1α,25-$(OH)_2D_3$ (31), but monocyte function as an APC appears be decreased (32, 33).

Over the past years in vitro methods have been described to differentiate dendritic cells (DC) from blood monocytes by in vitro culture with GM-CSF, IL-4 (34–36), or IL-13 (37). These cultured DC show functional and phenotypic characteristics typical of the immature stage of differentiation (i.e., high capacity of Ag uptake and processing, low capacity to stimulate T cell proliferation) and can be further differentiated in vitro into mature DC with TNF-α, LPS, IL-1, or CD40L (35, 38). As they are the most potent APC in vitro and in vivo, DC play a key role in the initiation of the immune response and are considered promising tools and targets for immunotherapy (39–41). It is therefore important to identify factors that might affect their process of differentiation and maturation (42, 43).

The aim of our work was to study the effects of 1α,25-$(OH)_2D_3$ on human monocyte-derived dendritic cell differentiation, maturation, and functional activities. Our results demonstrate that 1α,25-$(OH)_2D_3$ inhibited DC differentiation and maturation into potent APC. Moreover, D_3-DC promoted the onset of a nonspecific hyporesponsivity in T cells. These findings may have relevance in the development of new therapeutic treatments in the field of transplants and autoimmune diseases

Materials and Methods

Cytokines and reagents

Human recombinant GM-CSF (sp. act., 1.1×10^4 U/mg) was obtained from Novartis (Basel, Switzerland). Human rIL-4 (sp. act., $>2 \times 10^6$ U/mg) and human rTNF-α (sp. act., $>2 \times 10^7$ U/mg) were obtained from PeproTech (London, U.K.). 1,25$(OH)_2D_3$ was purchased from Sigma (St. Louis, MO).

*Laboratory of Experimental Surgery, Surgical Department, and †Department of Pathology, San Raffaele Scientific Institute, Milan, Italy; ‡University of Milan, Milan, Italy; §Department of Immunology and Cell Biology, Mario Negri Institute, Milan, Italy

Received for publication October 12, 1999. Accepted for publication February 11, 2000.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by grants from the National Research Center (Finalized Project Biotechnology 97.01301.PF 49) and Istituto Superiore di Sanità, Italy.

[2] Address correspondence and reprint requests to Dr. Lorenzo Piemonti, Laboratory of Experimental Surgery, San Raffaele Scientific Institute, Via Olgettina 60, 20132 Milan, Italy. E-mail address: piemonti.lorenzo@hsr.it

[3] Abbreviations used in this paper: 1α,25-$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; DC, dendritic cells; CD40L, CD40 ligand; MR, mannose receptor; TT, tetanus toxin; $_3$-DC, DC differentiated in the presence of 10 nM 1α,25-$(OH)_2D_3$; ctr-DC, control DC; LY, Lucifer Yellow.

Copyright © 2000 by The American Association of Immunologists

0022-1767/00/$02.00

Downloaded from http://journals.aai.org/jimmunol/article-pdf/164/9/4443/1118540/4443.pdf by guest on 14 May 2025

DC culture

Highly enriched monocytes (>80% $CD14^+$) were obtained from buffy coats of 20 blood donors (through the courtesy of Centro Trasfusionale, Ospedale San Raffaele, Milan, Italy) by Ficoll and Percoll gradients and were purified by adherence. Monocytes were cultured for 7 days at 1×10^6/ml in six-well tissue culture plates (Falcon, Becton Dickinson, Rutherford, NJ) in RPMI (Biochrom, Berlin, Germany) and 10% FCS (HyClone, Logan, UT) supplemented with 50 ng/ml GM-CSF and 10 ng/ml IL-4 and with (D_3-DC) or without (ctr-DC) various concentrations of 1α,25-$(OH)_2D_3$. In the control group (GM-CSF plus IL-4) the cell yield was about 80% of input cells. All cultures were tested for the presence of endotoxin (<0.03 U/ml; Lymulus test, BioWhittaker, Walkersville, MD).

DC maturation

LPS (10 ng/ml) was added to induce maturation of DC for at least 36 h of culture. Alternatively, J558L cells transfected with the ligand for CD40 (J558LmCD40L) were used to induce CD40 triggering on DC. Untransfected J558L cells were used as a control. After irradiation (10,000 rad) J558L cells were seeded together with DC at a 1:1 ratio in 24-well culture plates in culture medium (1×10^6 cells/well). Cells were recovered after 48–72 h of culture.

FACS analysis

Cell staining was performed using mouse mAbs followed by FITC-conjugated, affinity-purified, isotype-specific, goat anti-mouse Abs (Ancell, Bayport, MN). The following mAbs were used: L243 (IgG2a, anti-MHC class II), 32.2 (anti-CD32), and IV.3 (anti-CD64) from American Type Culture Collection (Manassas, VA); UCHM-1 (IgG2a, anti-CD14) and W6/32 (IgG2a, anti-MHC I) from Sigma; SK9 (IgG2b, anti-CD1a) from Becton Dickinson (San Jose, CA); B73.1 (IgG2a, anti-CD16) from Dr. G. Trinchieri (Philadelphia, PA); PAM-1 (IgG1 anti-mannose receptor) (44, 45); BB1 (IgM, anti-CD80), BU63 (IgG1, anti-CD86), and EA-5 (IgG1 anti-CD40) from Ancell; and HB15a (IgG2b, anti-CD83) from Immunotech (Marseilles, France). Results are expressed as the percentage of positive cells or as fluorescence intensity (FI), calculated according to the formula: FI = mean fluorescence (sample) − mean fluorescence (control).

Endocytosis

Mannose receptor (MR)-mediated endocytosis was measured as the cellular uptake of FITC-dextran and was quantified by flow cytometry. Approximately 2×10^5 cells/sample were incubated in medium containing FITC-dextran (1 mg/ml; m.w., 40,000; Sigma) for 0, 60, and 120 min. After incubation cells were washed twice with PBS to remove excess dextran and were fixed in cold 1% formalin. The quantitative uptake of FITC-dextran by the cells was determined by FACS. At least 8,000 cells/sample were analyzed. Fluid phase endocytosis through membrane ruffling was measured as the cellular uptake of 1 mg/ml of Lucifer Yellow (LY) dipotassium salt (Sigma) and was quantified by flow cytometry.

Mixed leukocyte reaction

DC cultured in GM-CSF and IL-4 and with or without 1α,25-$(OH)_2D_3$ for 7 days were extensively washed, irradiated (3000 rad from a ^{137}Cs source), and added in graded doses to 1×10^5 responder cells in 96-well flat-bottom Microtest plates (Costar, Cambridge, MA). Responder cells were purified allogeneic T cells depleted of autologous APC by passage with CD14- and CD19-coated Dynabeads (Unypath, Milan, Italy). Each group was performed in triplicate. Thymidine incorporation was measured on day 5 by a 16-h pulse with [^{3}H]thymidine (1 μCi/well; spec. act., 5 Ci/mmol; Amersham, Aylesbury, U.K.).

Ag presentation assay

Tetanus toxin (TT)-responsive T cell lines were generated in our laboratory by culturing mononuclear cells with TT (36 μg/ml; Cannaught, Willowdale, Canada) for 1 mo in the presence of IL-2. TT-responsive T cells were tested at least 2 wk after the last PBMC stimulation and 5 days after the last addition of IL-2. DC were obtained from the same donor by culturing monocytes. After 7 days DC were preincubated with TT (6 μg/ml) for 12 h and with LPS for 48 h. Then DC were extensively washed, irradiated (3000 rad), and cocultured with autologous TT-responsive T cell lines for 72 h in 96-well microtiter plates, and [^{3}H]thymidine uptake was measured during the last 12 h of culture (1 μCi/well; sp. act., 5 Ci/mmol; Amersham).

IL-12p70 measurement

After 7 days of culture with GM-CSF (50 ng/ml) and IL-4 (10 ng/ml) in the presence or the absence of 1α,25-$(OH)_2D_3$, DC were washed twice and cultured for 3 days at 0.5×10^6/ml in a 24-well flat-bottom plate (Costar). DC were either nonstimulated or stimulated with TNF-α (10 ng/ml), LPS (50 ng/ml), J558L cells transfected with CD40L (J558LmCD40L), or untransfected J558m cells. After 3 days medium was collected, and IL-12 p70 was quantified by ELISA (commercial kits from Endogen, Boston, MA).

Anergy assay

Allogeneic T cells were prepared from human blood using Ficoll and Percoll gradients and subsequent depletion of B cells and monocytes by plastic adherence and by Ab-coated immunomagnetic beads (Unipath, Milan, Italy) according to a standard protocol. T cells were cocultured during the first incubation at a density of 1×10^6/ml with 1×10^4/ml ctr-DC or D_3-DC (DC were prepared as described above and matured with LPS). Three days later T cells were separated by Ficoll gradient (Sigma), extensively washed, depleted of DC using Ab-coated immunomagnetic beads (Ab anti CD32 and MR), and rested for 5 days in medium alone. Subsequently, T cells were restimulated with mature ctr-DC generated from the same donor used in the first coculture or from another unrelated donor.

Electron microscopy

DC were processed for electron microscopy. DC were fixed for 2 h in 2.5% glutaraldehyde in 0.1 M cacodylate buffer. Then they were postfixed in 1% OsO_4 in cacodylate buffer at 4°C for 1 h, dehydrated in graded ethanol up to propylene oxide, and finally embedded in an Epon-Araldite mixture. Well-preserved areas were identified by light microscopy of semithin sections (0.5 mm). Subsequently, serial ultrathin sections (80 nm) were mounted on 200-mesh copper grids, stained with uranyl acetate and lead citrate, and finally examined with a Zeiss CEM 902 electron microscope (New York, NY).

Calculations and statistical analysis

Data were expressed as the mean ± SD. Comparisons were performed using Student's t test. A p value of <0.05 was considered statistically significant.

Results

1α,25-$(OH)_2D_3$ interferes with the differentiation of DC from human monocytes

To investigate the effect of 1α,25-$(OH)_2D_3$ on DC differentiation from monocytes, we cultured monocytes in the presence of GM-CSF, IL-4 (control group, ctr-DC), and various concentrations (0.5–100 nM) of 1α,25-$(OH)_2D_3$ (D_3-DC). 1,25$(OH)_2D_3$ did not affect cell recovery at any concentration tested. The standard concentration of 1α,25-$(OH)_2D_3$ chosen for the study was 10 nM, the highest concentration considered physiological (4); this was also used in previous studies for leukocyte differentiation (30). Upon culture with GM-CSF and IL-4 for 7 days cells became nonadherent and clustered, with abundant cytoplasm and protruding veils typical of DC. Despite a similar morphology (Fig. 1), the presence of 1α,25-$(OH)_2D_3$ in culture interfered with the differentiation of monocytes into DC. Fig. 2 shows a representative experiment of surface phenotype. Control cells expressed high levels of CD1a and were negative or low positive for CD14 and CD16, while with 1α,25-$(OH)_2D_3$ the cells were negative or low positive for CD1a but expressed higher levels of CD14. Analysis of MHC class I showed an up-regulation of D_3-DC, whereas expression of MHC II, CD40, and CD86 molecules was decreased. Ag uptake molecules, such as CD32 and MR, were increased. DC obtained after 7-day culture with GM-CSF and IL-4 could be further differentiated in vitro into fully mature DC by exposure to LPS or CD40L. D_3-DC were not sensitive to maturation stimuli. In fact, after exposure to LPS (Fig. 3) or CD40L, D_3-DC were unable to up-regulate CD83 as well as the molecules involved in Ag presentation (MHC I, MHC II, CD80, CD86, and CD40) and to down-regulate the Ag uptake molecules (CD2 and MR). A summary of six different experiments is shown in Table I.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/164/9/4443/1118540/4443.pdf by guest on 14 May 2025

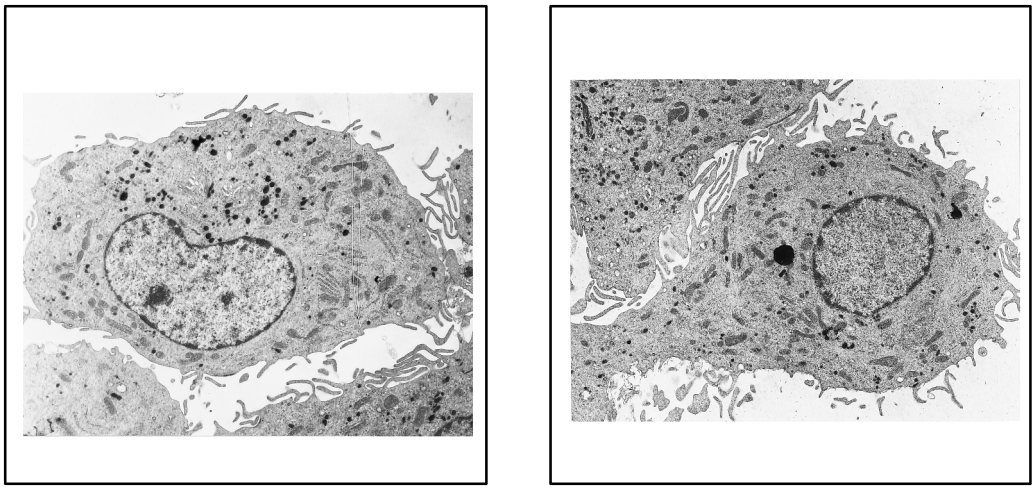


FIGURE 1. Morphological appearance at electron microscopy of D_3-DC and Ctr-DC. DC were differentiated from monocytes cultured for 7 days in GM-CSF (50 ng/ml) and IL-4 (10 ng/ml) in the absence (*left*) or the presence (*right*) of 1α,25-$(OH)_2D_3$ (10 nM).

DC differentiated in the presence of 1α,25-$(OH)_2D_3$ showed augmented Ag uptake capacity and inhibited immunostimulatory capacity

Immature DC, such as cells derived by culturing monocytes with GM-CSF and IL-4 for 7 days, express a potent ability to uptake external molecules, essentially via two main mechanisms: a receptor-mediated endocytosis and a fluid phase endocytosis (macropinocytosis). To study the endocytic capacity of D_3-DC, we used two fluorescent markers: LY, a nonspecific fluid phase marker, and FITC-DX, which is mainly taken up via the MR. DC cultured with GM-CSF and IL-4 in the presence of 1α,25-$(OH)_2D_3$ showed a vigorous endocytosis of FITC-dextran, higher than control DC (Fig. 4*A*). The same behavior was seen when we used LY as marker of fluid phase pinocytosis (Fig. 4*B*). DC are potent stimulators of allogeneic T cells. We tested whether D_3-DC were able to stimulate allogeneic T lymphocytes in MLR. D_3-DC showed very little ability to induce allogeneic T lymphocyte proliferation (Fig. 5*A*). Moreover, the immunostimulatory capacity of D_3-DC in

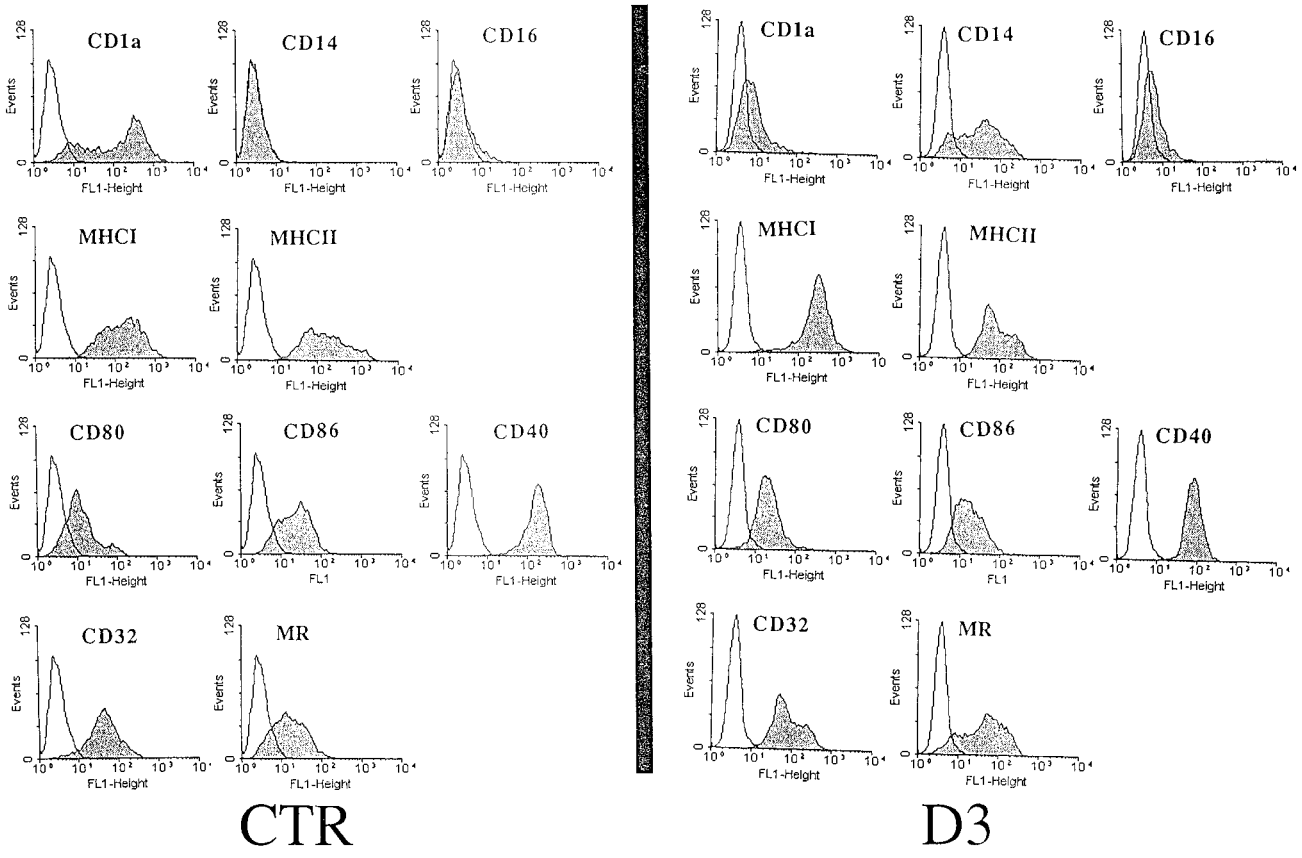


FIGURE 2. Flow cytometric analysis of molecules expressed by DC differentiated in the presence of 1α,25-$(OH)_2D_3$. Monocytes were cultured for 7 days with 50 ng/ml GM-CSF and 10 ng/ml IL-4 in the presence (D_3-DC) or the absence (ctr-DC) of 1α,25-$(OH)_2D_3$. Cells were labeled with the designed mAb and then with FITC-labeled goat anti mouse-Ig. Data shown are representative of six experiments.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/164/9/4443/1118540/4443.pdf by guest on 14 May 2025

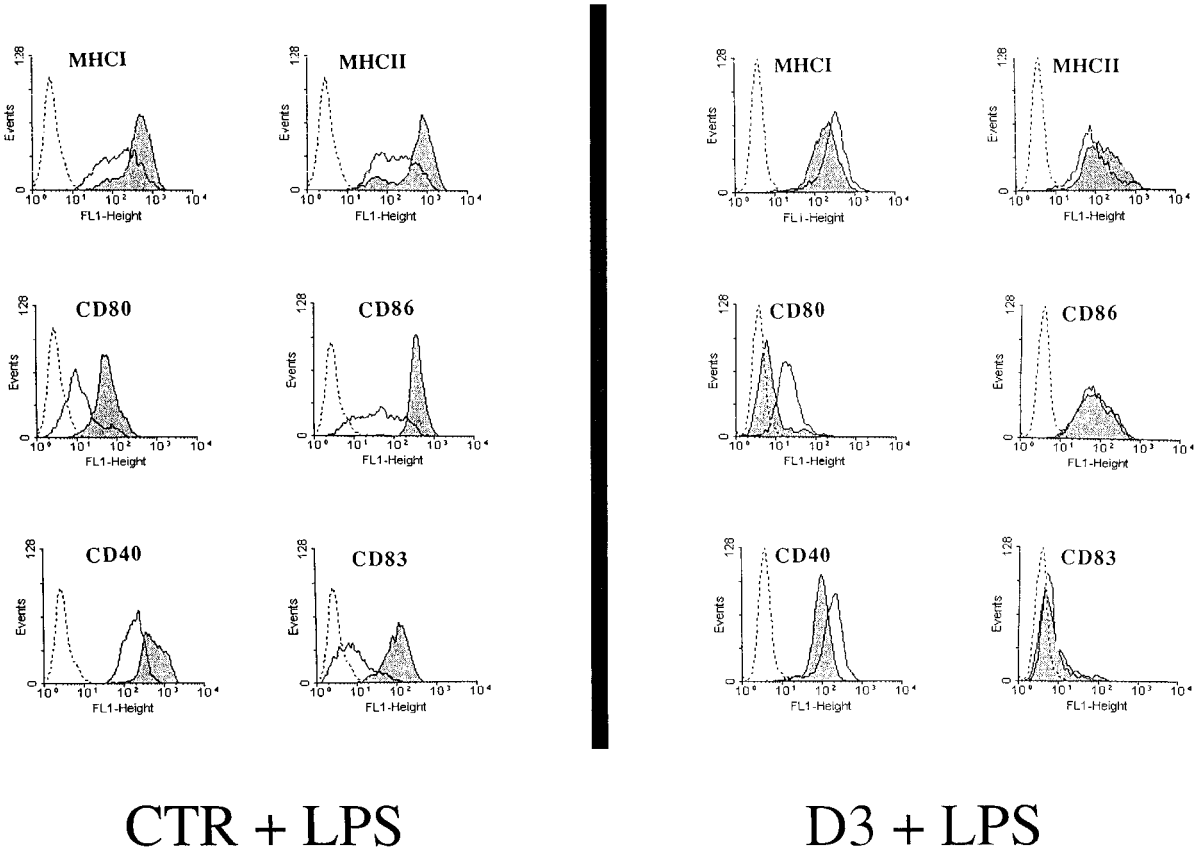


FIGURE 3. $1\alpha,25\text{-}(OH)_2D_3$ differentiated DC are not sensitive to maturation with LPS. Monocytes were cultured for 7 days in RPMI and 10% FCS supplemented with 50 ng/ml GM-CSF and 10 ng/ml IL-4 in the presence (D_3-DC) or the absence (ctr-DC) of $1\alpha,25\text{-}(OH)_2D_3$. Then DC were cultured for 48 h with LPS (1 μg/ml). Cells were labeled with the designed mAb and then with FITC-labeled goat anti mouse-Ig. Dotted line, isotype negative control; white area, immature DC; dashed area, after exposition to LPS.

Table I. *Phenotype analysis of DC differentiated in the presence of $1\alpha,25\text{-}(OH)_2D_3$*[a]

	Ctr-DC	D_3-DC	Ctr-DC + LPS	D_3-DC + LPS	Ctr DC + CD40L	D_3-DC + CD40L
Myeloid Ags						
CD68	0.35	0.5	NT	NT	NT	NT
CD14	3	26	NT	NT	NT	NT
Presentation molecules						
MHCII	181	142	480	120	465	223
MHCI	138	217	403	277	405	177
CD1a	80	19	NT	NT	NT	NT
Costimulatory/signaling molecules						
CD80	23	26.2	58	21	56	29
CD86	45	29	233	24	111	38
CD40	157	84	270	168	327	161
Potential Ag uptake receptors						
CD16	5.6	9.5	NT	NT	NT	NT
CD32	54	100	NT	NT	NT	NT
CD64	0.5	0.6	NT	NT	NT	NT
MR	57	102	23	83	39	84
Maturation Ag						
CD83	7	8	67	7	80	20

[a] DC were differentiated from monocytes with GM-CSF (50 ng/ml) and IL-4 (10 ng/ml) with (Ctr-DC) or without $1\alpha,25\text{-}(OH)_2D_3$ vitamin (D_3-DC) for 7 days. Maturation was induced by culture with LPS (10 ng/ml) or CD40L-transfected cell line (J558LmCD40L) for 48 h. Results are expressed as fluorescence intensity (FI). Data were expressed as mean (n = 6). NT, not tested.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/164/9/4443/1118540/4443.pdf by guest on 14 May 2025

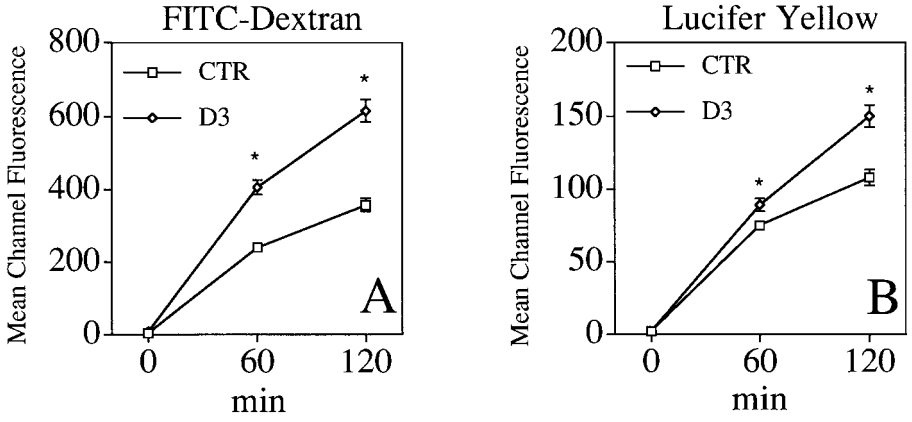


FIGURE 4. Endocytic activity of DC differentiated in the presence of 1α,25-$(OH)_2D_3$. Monocytes were cultured for 7 days with 50 ng/ml GM-CSF and 10 ng/ml IL-4 in the presence (D_3-DC) or the absence (ctr-DC) of 1α,25-$(OH)_2D_3$. Endocytosis was evaluated as uptake of 1 mg/ml FITC-DX (*A*) or 1 mg/ml LY (*B*) and was measured using FACS. Results are expressed as fluorescence intensity (FI) (n = 6). *, $p < 0.05$.

MLR was not increased by LPS or was increased to a much lower extent by CD40L, compared with that of ctr-DC (Fig. 5). In view of the fact that Ag capture was increased in D_3-DC but the stimulatory capacity was impaired in MLR, we evaluated the ability to present soluble Ag that need to be taken up and processed. Cells differentiated in the presence of 1α,25-$(OH)_2D_3$ showed much lower efficiency in presenting TT to specific autologous T cell lines (Fig. 6).

1α,25-$(OH)_2D_3$ affects IL-12 p70 production by DC

To investigate the capacity of 1α,25-$(OH)_2D_3$ to interfere with IL-12 production, after 7 days of culture with GM-CSF and IL-4 with or without 1α,25-$(OH)_2D_3$, DC were washed, seeded in the presence of maturation-inducing stimuli, and cultured for 3 days. Supernatants were quantified for IL-12 p70. IL-12 p70 production was significantly decreased when D_3-DC were exposed to TNF-α (53.5 vs 72.2 pg/0.5 $\times$ 10^6 cells/ml; $p = 0.05$) or LPS (45.7 vs 79.8 pg/0.5 $\times$ 10^6 cells/ml; $p = 0.02$), or CD40L (38.6 vs 481.7 pg/0.5 $\times$ 10^6 cells/ml; $p = 0.002$; Fig. 6).

Induction of hyporesponsivity in T cells by 1α,25-$(OH)_2D_3$-treated DC

As T cell stimulation via TCR in the absence of a second signal by costimulatory molecules and/or secreted cytokines may induce a state of hyporesponsivity or anergy, we tested whether D_3-treated DC induced an alloantigen-specific tolerance. In these experiments allogeneic T cells were first cocultured with ctr-DC or D_3-DC and exposed to LPS for 3 days. Then cells were extensively washed, depleted of remaining DC by MR or CD2 using Immuno-Dynabeads (Unipath, Milan, Italy), and rested for 5 days. T cells (viability, >90%) were then restimulated in a second coculture with mature ctr-DC. T cells first cocultured with ctr-DC responded vigorously to restimulation with mature ctr-DC. In contrast, T cells first cocultured with D_3-DC were hyporesponsive to further stimulation with ctr-DC (Fig. 7*A*). To determine whether this hyporesponsivity was alloantigen specific, the rescued T cells were restimulated with DC generated from an unrelated donor. In this case also, T cells showed a profound inhibition of proliferative capacity compared with T cells cocultured with untreated DC (Fig. 7*B*). These results indicate that D_3-Dc exposed to LPS induce a state of hyporesponsivity in T cells, which was not alloantigen restricted. Of interest, when immature D_3-DC were used as stimulator in the first coculture, T cells were not inhibited (data not shown).

Effects of 1α,25-$(OH)_2D_3$ on already differentiated immature DC

To evaluate the effects of 1α,25-$(OH)_2D_3$ on differentiated immature DC, monocytes were cultured for 7 days in the presence of GM-CSF and IL-4. Cells were then washed and incubated again with IL-4, GM-CSF, and 1α,25-$(OH)_2D_3$ for 3 or 7 additional days. Control cells were incubated with GM-CSF and IL-4 for the same period of time. Treatment with 1α,25-$(OH)_2D_3$ partially reversed DC differentiation, as demonstrated by a down-regulation of CD1a and an up-regulation of CD14; expression of CD80, CD86, and MHC I was not affected. In contrast, MHC II and CD40 were significantly down-regulated (Fig. 8). After exposure to LPS or CD40L, D_3-DC showed a lower expression of MHC I, MHC II,

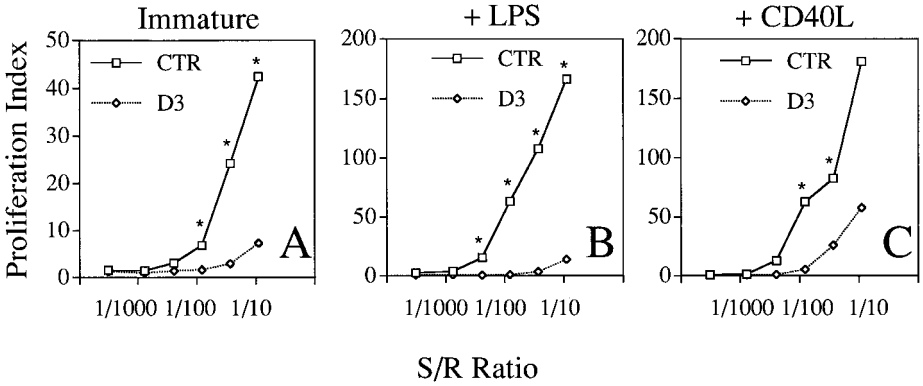


FIGURE 5. Effect of 1α,25-$(OH)_2D_3$ on the stimulatory activity in MLR. Monocytes were cultured with GM-CSF and IL-4 in the presence (D_3-DC) or the absence (ctr-DC) of 1α,25-$(OH)_2D_3$. After 7 days DC were extensively washed, irradiated (3000 rad), and added in graded doses to 1 $\times$ 10^5/well purified allogeneic responder T cells in 96-well flat-bottom Microtest plates (immature). In a second type of experiment, immature DC were further cultured for 48 h with LPS (10 ng/ml) or CD40L-transfected cells. Responder cells were allogeneic T cells depleted of autologous APC. Each group was tested in triplicate. Thymidine incorporation was measured on day 5 by a 16-h pulse with [^{3}H]thymidine (n = 6). *, $p < 0.05$.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/164/9/4443/1118540/4443.pdf by guest on 14 May 2025

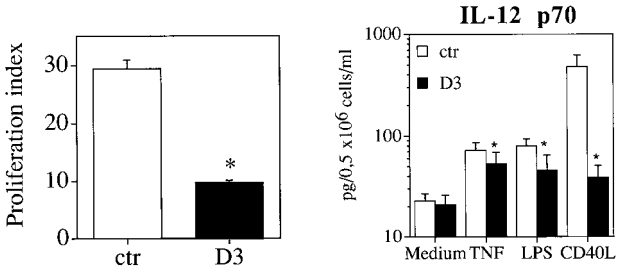


FIGURE 6. $1\alpha,25\text{-}(OH)_2D_3$ inhibits the presentation of soluble Ag and affects IL-12 production by DC. *Left*, Monocytes were cultured with GM-CSF and IL-4 in the absence (ctr-DC) or the presence of $1\alpha,25\text{-}(OH)_2D_3$ (D_3-DC). After 7 days, DC were pulsed with TT (6 μg/ml) for 12 h. After Ag pulsing, DC were further cultured for 48 h in medium with LPS (50 ng/ml). Cells were then extensively washed and mixed 1/10 with 1×10^5/well TT-specific T cell lines. Proliferation was assessed as [^{3}H]TdR uptake during the last 18 h of a 3-day experiment ($n = 3$). *, $p < 0.05$. *Right*, After 7 days of culture with GM-CSF and IL-4 and with (D_3-DC) or without (ctr-DC) $1\alpha,25\text{-}(OH)_2D_3$, DC were washed and stimulated at 0.5×10^6/ml with LPS (1 μg/ml), TNF-α (10 ng/ml), or CD40L. Control groups were DC cultured in medium alone. Supernatants were harvested 48 h later and tested for IL-12 p70. Result are expressed as picograms per 0.5×10^6 cells/ml and are the mean of four experiments ($n = 4$). *, $p < 0.05$.

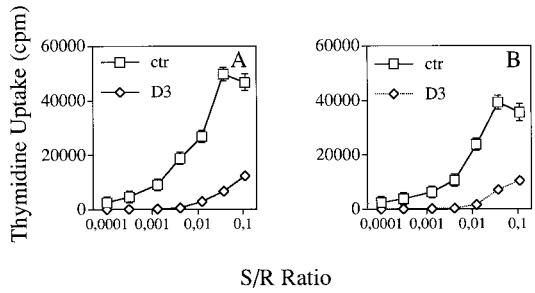


FIGURE 7. Induction of T cell hyporesponsivity by $1\alpha,25\text{-}(OH)_2D_3$-treated DC. Purified T cells (1×10^6) were cultured for 3 days with DC (1×10^4) differentiated in medium containing $1\alpha,25\text{-}(OH)_2D_3$ (10 ng/ml; D_3-DC) or DC control (ctr-DC). Both groups of APC were matured with LPS. After this first coculture T cells were rescued, washed, depleted of residual DC, rested for 5 days in medium alone, and then restimulated in a second coculture with untreated mature DC, generated either from the same donor (*A*) or from a second, unrelated donor (*B*). Thymidine incorporation was measured after 48 h ($n = 4$).

CD80, CD86, CD83, and CD40. A summary of four experiments is shown in Table II. Finally, we evaluated the influence of $1\alpha,25\text{-}(OH)_2D_3$ on the endocytic activity of immature DC and on the capacity to stimulate T lymphocytes in MLR. $1\alpha,25\text{-}(OH)_2D_3$ significantly increased the uptake of FITC-dextran and Ag uptake receptor expression, while the capacity to stimulate allogeneic T lymphocytes was decreased compared with that of untreated DC (data not shown). Overall, these results demonstrate that $1\alpha,25\text{-}(OH)_2D_3$ impaired the maturation of DC even when added tn already differentiated cells.

Discussion

It is well known that the active form of vitamin D, $1\alpha,25\text{-}(OH)_2D_3$, modulates lymphocyte and macrophage functions (46). We demonstrated a new target of $1\alpha,25\text{-}(OH)_2D_3$ action on the immune system: DC. Because DC have the unique property to activate naive T cells and are required for the induction of a primary response (47), the suppression of DC function may very efficiently control the specific immune response (48). $1\alpha,25\text{-}(OH)_2D_3$ showed complex effects on DC. $1\alpha,25\text{-}(OH)_2D_3$ partially blocked the GM-CSF- and IL-4-driven differentiation of monocytes to DC. In fact, in the presence of $1\alpha,25\text{-}(OH)_2D_3$, despite a quite similar morphology, the expression of CD1a was inhibited and CD14 expression was increased, a marker of monocytes/macrophages normally not present on DC (49). In previous studies high expression of CD14 was found in monocyte-derived DC cultured in the presence of immunosuppressive factors such as glucocorticoid and IL-10 (50, 51). The intensity of CD14 expression was lower in vitamin D_3-derived DC than in glucocorticoid-derived or IL-10-derived DC, and CD14 was not seen with low concentration (5%) of FCS (data not shown) Despite the persistence of CD14, other markers of monocytes/macrophages, such as CD16 and CD68, were not present in vitamin D_3-treated DC. Therefore, we conclude that vitamin D_3 inhibits a full differentiation of monocytes

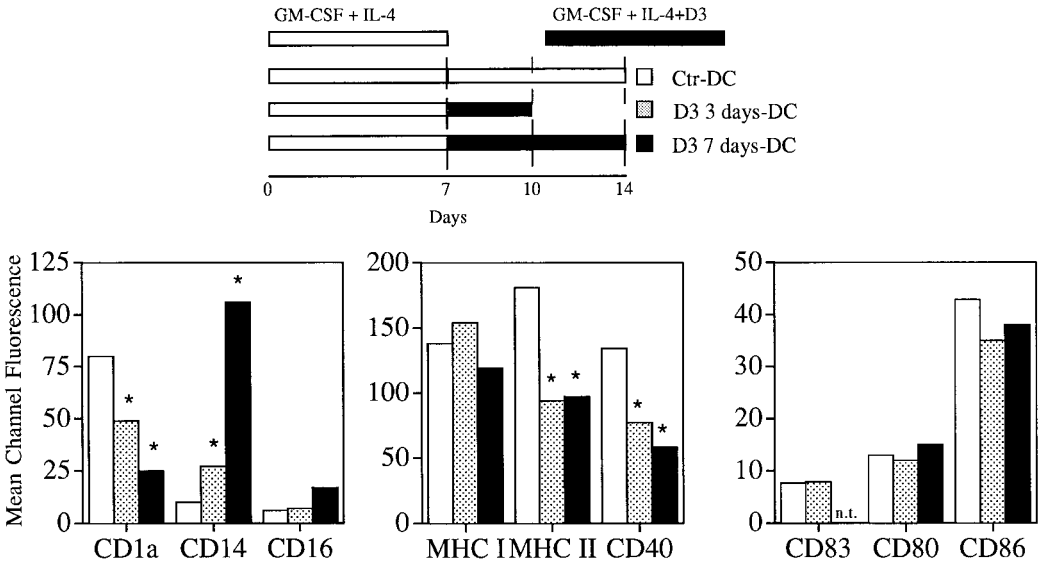


FIGURE 8. $1\alpha,25\text{-}(OH)_2D_3$ partially reversed DC differentiation. Immature DC were differentiated from monocytes by a 7-day culture in the presence of GM-CSF (50 ng/ml) and IL-4 (10 ng/ml), then cells were washed and additionally incubated in the presence of IL-4 and GM-CSF (ctr) or IL-4, GM-CSF, and $1\alpha,25\text{-}(OH)_2D_3$ (D_3) for 3 or 7 days. Cells were labeled with the designed mAb and then with FITC-labeled goat anti-mouse Ig. Results are expressed as fluorescence intensity (FI), calculated according to the formula: FI = mean fluorescence sample − mean fluorescence control ($n = 4$). *, $p < 0.05$.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/164/9/4443/1118540/4443.pdf by guest on 14 May 2025

Table II. *Phenotype analysis of immature DC after exposure to LPS and CD40L in presence of 1α,25-$(OH)_2D_3$*[a]

	Ctr + LPS	D_3 + LPS	Ctr + CD40L	D_3 + CD40L
Presentation molecules				
MHCII	369	273	396	221
MHCI	388	221	377	297
Costimulatory/signaling molecules				
CD80	74	48	82	58
CD86	NT	NT	100	60
CD40	290	221	369	232
Maturation Ag				
CD83	29	8.7	36	18

[a] Immature DC obtained after 7-day culture with GM-CSF and IL-4 were treated with 1α,25-$(OH)_2D_3$. After 72 h, cells were exposed to maturation-inducing stimuli LPS (10 ng/ml) or J558mCD40L for 48 h. Results are expressed as fluorescence intensity (FI). Data were expressed as mean ($n = 4$). NT, not tested.

into DC, but, unlike IL-10, does not promotes differentiation toward macrophages. In the past, a clear effect of D_3 has been shown on monocytes, bone marrow precursors, and monocytic leukemic cell lines on the differentiation toward macrophages, but under different culture conditions (28, 30, 52, 53). 1α,25-$(OH)_2D_3$ is also important in osteoclast generation, defining the commitment of monocytes differentiating into osteoclasts as a cooperative associative mechanism involving osteoblastic cells (54, 55). Monocytes may be considered relatively immature precursors with multiple differentiation potentials that depend upon the microenvironment (56). Our data showed that similar to cytokines such as M-CSF, GM-CSF, TGF-β, and IL-4, the hormone 1α,25-$(OH)_2D_3$ may play an important role in the final decision determining whether monocytes will acquire DC, macrophage, or osteoclastic characteristics and functions, in particular inhibiting DC differentiation.

1α,25-$(OH)_2D_3$-treated DC showed other important modifications in phenotype. Normally we can identify two major phases in the life of DC (40, 41, 57, 58): an immature stage characterized by a high efficiency in taking up and processing Ags associated with high expression of molecules involved in Ag uptake as MR, such as CD32; and a mature stage in which the Ag uptake capacity is lost, the cell migrates toward regional lymph nodes, and the function shifts to become a potent APC (59, 60) associated with a high expression of molecules involved in Ag presentation and T cell stimulation, such as MHC I, MHC II, CD80, CD86, CD40, and CD83. Exposure of differentiating monocytes to 1α,25-$(OH)_2D_3$ increased the expression of molecules involved in Ag capture (CD32, MR), while some important costimulatory molecules (CD86, CD40) were inhibited. This phenotype correlates with impaired Ag-presenting function for T lymphocytes and higher endocytic activity. Moreover, 1α,25-$(OH)_2D_3$ strongly inhibited DC maturation, as demonstrated by a low or absent increase in the expression of MHC I, MHC II, CD80, CD86, CD40, and CD83 and by the impaired stimulatory capacity for T lymphocytes after exposure to LPS or CD40L. Finally, as recently reported with already differentiated DC (61), D_3-DC showed impaired IL-12 production after CD40L, LPS, or TNF-α exposure. These results extend the immunosuppressive effects of this hormone and confirm a role for 1α,25-$(OH)_2D_3$ as a regulator of immune cell differentiation and function.

The effects of 1α,25-$(OH)_2D_3$ on immature DC that have been differentiated for 7 days in the presence of GM-CSF and IL-4 appear to be similar to, but not identical with, the effects of 1α,25-$(OH)_2D_3$ included at the beginning of the culture. Overall, the effects can be summarized as follows: a partial conversion to a monocyte/macrophage phenotype, an impaired capacity to reach maturation, and a decreased ability to stimulate T cells (the latter not shown). These results confirm the in vitro instability of immature DC generated with GM-CSF and IL-4 (56, 62). Palucka et al. (56) showed that upon removal of both GM-CSF and IL-4 and/or reculture with M-CSF, immature $CD1a^+/CD14^-$ DC easily converted to a macrophage phenotype expressing CD14 with a decreased ability to stimulate allogeneic T cells. Thus 1α,25-$(OH)_2D_3$ acts at two different steps of DC life: 1) inhibiting the differentiation from monocytic precursors and thus impairing the normal turnover of DC in tissues, and 2) inhibiting the terminal maturation of DC into a potent APC.

The inhibitory effect of 1α,25-$(OH)_2D_3$ on DC maturation and differentiation is very similar to that of IL-10, an anti-inflammatory cytokine, and to that of glucocorticoids. In fact, both IL-10 and glucocorticoids were shown to prevent monocyte differentiation and maturation to DC, to impair IL-12 production, and to increase Ag uptake (43, 50, 51).

Of interest is the fact that 1α,25-$(OH)_2D_3$-differentiated DC matured with LPS or CD40L induced hyporesponsivity of allogeneic T cells. T cells cocultured with D_3-DC showed impaired proliferation to a second stimulation with control DC from the same as well as from an unrelated donor. A direct effect of D_3 on T cells is excluded, as D_3-DC were extensively washed before coculture. Moreover, T cell viability before the second stimulation was >90%, and T cells were able to proliferate upon addition of exogenous IL-2 (data not shown). The induction of T cell anergy has been reported by Steinbrink et al. (63) with DC differentiated in the presence of IL-10, but several differences can be outlined, showing different mechanisms of action between IL-10 and D_3. In IL-10-treated DC the induction of anergy is associated only with immature DC and is alloantigen specific. In our work it was not alloantigen specific and was not observed when we used immature D_3-DC. It appeared at least in part linked to a soluble factor(s) secreted by D_3-DC exposed to LPS. In fact, supernatants from these cells, when added in a first coculture to control mature DC and allogeneic T cells, inhibited T cell proliferation, while supernatants from immature D_3-DC or mature ctr-DC did not. The generation of DC able to induce T cell hyporesponsivity might be a first step in the development of treatments for patients at risk of transplant rejection or with autoimmune or allergic diseases. The therapeutic use of these cells, however, requires further studies, as the hyporesponsivity was not specific, and putative factors involved in the induction of T cell hyporesponsivity remain to be defined.

The effects described in our work were seen in a range of 1α,25-$(OH)_2D_3$ concentrations from 5×10^{-11} to 10^{-7}. Importantly, calcitriol is effective at concentrations that are considered physiological (10^{-10}–10^{-8} mol/l) (64) and that correspond to the accepted affinity value for its receptor (65). The physiological role of vitamin D in immune responses is not clearly defined. In vivo, both an excess and a deficiency of vitamin D suppress the delayed hypersensitivity response (66) or Ig production (67). Vitamin D-deficient animals and humans have a higher risk of infection, probably related to impaired macrophage function (68). The monocyte function as APC seem to be decreased (32). The NK cell activity is enhanced by 1α,25-$(OH)_2D_3$. This enhancing effect of the nonspecific immune defense contrasts with an inhibition of the Ag-specific immune response, as demonstrated by decreased T cell proliferation and activity (decreased IL-2, IFN-γ, GM-CSF synthesis and secretion). It is very difficult to clarify the endocrine activity of 1α,25-$(OH)_2D_3$ in the immune system. A possible paracrine or autocrine activity may be postulated. The secretory role of

Downloaded from http://journals.aai.org/jimmunol/article-pdf/164/9/4443/1118540/4443.pdf by guest on 14 May 2025

macrophages may be central to the production of localized concentration of 1α,25-$(OH)_2D_3$ within immune microenvironments. Normal macrophages have been shown to synthesize 1α,25-$(OH)_2D_3$ when activated by agents such as IFN-γ and LPS (1, 4). In granulomatous disorders such as sarcoidosis and tuberculosis, macrophages are able to produce 1α,25-$(OH)_2D_3$ and appear to be insensitive to feedback control by 1α,25-$(OH)_2D_3$ itself as well as other regulators, such as calcium and parathyroid hormone. It is tempting to speculate that 1α,25-$(OH)_2D_3$ produced by macrophages, by inhibiting differentiation and function of DC, may contribute to the peripheral anergy in sarcoidosis and to the persistence of granulomatous lesion in tuberculosis.

Another site of interest for 1α,25-$(OH)_2D_3$ action is the skin. It is likely that the major source of vitamin D for human is not dietary, but results from its manufacture by a chemical photolysis reaction in skin. Vitamin D_3 itself is a biologically inert molecule. It must be activated by 25-hydroxylation in the liver to produce the major circulating form of vitamin D, 25-hydroxyvitamin D_3. However, 25-hydroxyvitamin D_3 is also biologically inactive at physiological concentrations, and it is finally activated in the proximal convoluted tubule cells of the kidney to produce 1α,25-$(OH)_2D_3$ (8). Keratinocytes, the most important cells of the skin, possess both the 24- and 1α-hydroxylase enzymes and thus can produce small amount of 1α,25-$(OH)_2D_3$. Therefore, UV exposure induces a systemic and a local increase in 1α,25-$(OH)_2D_3$ in the skin. It is known that after UV exposure, Langerhans cells (epidermal $CD1a^+$ cells) disappear from the healthy skin, and $CD11b^+$ macrophage-like cells appear in few days (69). Moreover, UV radiation induces apoptosis and suppresses the immune function of epidermal Langerhans cells (70). Although other cytokines, such as IL-10, are known to play an important role in UV-induced immunosuppression (71), it is tempting to speculate that 1α,25-$(OH)_2D_3$ could also contribute to some of the modifications of Langerhans cells and could be responsible for the decrease in DC in skin after UV exposition.

In conclusion, our data suggest that 1α,25-$(OH)_2D_3$ may modulate the immune system, acting at the very first step of the immune response through the inhibition of DC differentiation and maturation into potent APC.

Acknowledgments

We thank Dr. Luciano Adorini (Roche, Milan, Italy) for stimulating discussion.

References

1. Manolagas, S. C., F. G. Hustmyer, and X. P. Yu. 1990. Immunomodulating properties of 1,25-dihydroxyvitamin D_3. *Kidney Int. 38(Suppl. 29):S-9.*
2. Manolagas, S. C., D. M. Provvedini, and C. D. Tsoukas. 1985. Interaction of 1,25-dihydroxyvitamin D_3 and the immune system. *Mol. Cell. Endocrinol. 43: 113.*
3. Lemire, J. M. 1995. Immunomodulatory actions of 1,25-dihidroxivitamin D_3. *J. Steroid Biochem. Mol. Biol. 53:599.*
4. Hewison, M. 1992. Vitamin D and the immune system. *J. Endocrinol. 132:173.*
5. Fournier, C., P. Gepner, M. Sadouk, and J. Charreire. 1990. In vivo beneficial effects of cyclosporine A and 1,25-dihydroxyvitamin D_3 on the induction of experimental autoimmune thyroiditis. *Clin. Immunol. Immunopathol. 54:53.*
6. Lemire, J. M., and D. C. Archer. 1991. 1,25-Dihydroxyvitamin D_3 prevents the in vivo induction of murine experimental autoimmune encephalomyelitis. *J. Clin. Invest. 87:1103.*
7. Cantorna, M. T., C. E. Hayes, and H. F. DeLuca. 1996. 1,25-Dihydroxyvitamin D_3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. *Proc. Natl. Acad. Sci. USA* 93:7861.
8. Hayes, C. E., M. T. Cantorna, and H. F. DeLuca. 1997. Vitamin D and multiple sclerosis. *Proc. Soc. Exp. Biol. Med. 216:21.*
9. Branisteanu, D. D., M. Waer, H. Sobis, S. Marcelis, M. Vandeputte, and R. Bouillon. 1995. Prevention of murine experimental allergic encephalomyelitis: cooperative effects of cyclosporine and 1α,25$(OH)_2D_3$. *J. Neuroimmunol. 61: 151.*
10. Lillevang, S. T., J. Rosenkvist, C. B. Andersen, S. Larsen, E. Kemp, and T. Kristensen. 1992. Single and combined effects of the vitamin D analogue KH1060 and cyclosporine A on mercuric-chloride-induced autoimmune disease in the BN rat. *Clin. Exp. Immunol. 88:301.*
11. Branisteanu, D. D., P. Leenaerts, B. Van Damme, and R. Bouillon. 1993. Partial prevention of active Heymann nephritis by 1α,25-dihydroxyvitamin D_3. *Clin. Exp. Immunol. 94:412.*
12. Hattori, M. 1990. Effect of 1α,25(OH)2D_3 on experimental rat nephrotoxic serum nephritis. *Nippon Jinzo Gakkai Shi. 32:147.*
13. Inaba, M., Y. Nishizawa, K. Song, H. Tanishita, S. Okuno, T. Miki, and H. Morii. 1992. Partial protection of 1α-hydroxyvitamin D_3 against the development of diabetes induced by multiple low dose streptozotocin injection in CD-1 mice. *Metabolism 41:631.*
14. Mathieu, C., J. Laureys, H. Sobis, M. Vandeputte, M. Waer, and R. Bouillon. 1992. 1,25-Dihydroxyvitamin D_3 prevents insulitis in NOD mice. *Diabetes 41: 1491.*
15. Mathieu, C., M. Waer, J. Laureys, O. Rutgeerts, and R. Bouillon. 1994. Prevention of autoimmune diabetes in NOD mice by 1,25-dihydroxyvitamin D_3. *Diabetologia 37:552.*
16. Casteels, K., M. Waer, R. Bouillon, K. Allewaert, J. Laureys, and C. Mathieu. 1996. Prevention of type I diabetes by late intervention with non hypercalcemic analogues of vitamin D_3 in combination with cyclosporine A. *Transplant. Proc. 28:3095.*
17. Jordan, S. C., R. Shibuka, and Y. Mullen. 1988. 1,25 Dihydroxyvitamin D_3 prolongs skin graft survival in mice. In *Vitamin D: Molecular, Cellular and Clinical Endocrinology*. A. W. Norman, K. Schaefer, H.-G. Grigoleit, and D. von Herrath, eds. Walter de Gruyter, Berlin, p. 346.
18. Jordan, S. C., M. Nigata, and Y. Mullen. 1988. 1,25 Dihydroxyvitamin D_3 prolong rat cardiac allograft survival. In *Vitamin D: Molecular, Cellular and Clinical Endocrinology*. A. W. Norman, K. Schaefer, H.-G. Grigoleit, and D. von Herrath, eds. Walter de Gruyter, Berlin, p. 334.
19. Lemire, J. M., D. C. Archer, A. Khulkarni, A. Ince, M. R. Uskokovic, and S. Stepkowski. 1992. Prolongation of the survival of murine cardiac allografts by the vitamin D_3 analogue 1,25-dihydroxy-delta 16-cholecalciferol. *Transplantation 54:762.*
20. Veyron, P., R. Pamphile, L. Binderup, and J.-L. Touraine. 1993. Two novel vitamin D analogues, KH1060 and CB 966, prolong skin allograft survival in mice. *Transplant. Immunol. 1:72.*
21. Provvedini, D. M., C. D. Tsoukas, L. J. Deftos, and S. C. Manolagas. 1983. 1,25-Dihydroxyvitamin D_3 receptors in human leukocytes. *Science 221:1181.*
22. Bhalla, A. K., E. P. Amento, T. L. Clemens, M. F. Holick, and S. M. Krane. 1989. Specific high-affinity receptors for 1,25-dihydroxyvitamin D_3 in human peripheral blood mononuclear cells: presence in monocytes and induction in T lymphocytes following activation. *J. Clin. Endocrinol. Metab. 68:774.*
23. Tsoukas, C. D., D. M. Provvedini, and S. C. Manolagas. 1984. 1,25-Dihydroxyvitamin D_3: a novel immunoregulatory hormone. *Science 224:1438.*
24. Tsoukas, C. D., D. Watry, S. S. Escobar, D. M. Provvedini, C. A. Dinarello, F. G. Hustmyer, and S. C. Manolagas. 1989. Inhibition of interleukin-1 production by 1,25-dihydroxyvitamin D_3. *J. Clin. Endocrinol. Metab. 69:127.*
25. Lemire, J. M., J. S. Adams, R. Sakai, and S. C. Jordan. 1984. 1,25-Dihydroxivitamin D_3 suppresses proliferation and immunoglobulin production by normal human peripheral blood mononuclear cells. *J. Clin. Invest. 74:657.*
26. Rigby, W. F. C., S. Denome, and M. W. Fanger. 1987. Regulation of lymphokine production and human T lymphocyte activation by 1,25-dihydroxyvitamin D_3. *J. Clin. Invest. 79:1659.*
27. Koeffler, H. P., T. Amatruda, N. Ikekawa, Y. Kobayashi, and H. F. DeLuca. 1984. Induction of macrophage differentiation of human normal and leukemic myeloid stem cells by 1,25-dihydroxyvitamin D_3 and its fluorinated analogues. *Cancer Res. 44:5624.*
28. Abe, E., C. Miyaura, H. Sakagami, M. Takeda, K. Konno, T. Yamazaki, S. Yoshiki, and Suda, T. 1981. Differentiation of mouse myeloid leukemia cells induced by 1,25-dihydroxyvitamin D_3. *Proc. Natl. Acad. Sci. USA 78:4990.*
29. Tanaka, H., E. Abe, C. Miyaura, T. Kuribayashi, K. Konno, Y. Nishii, and T. Suda. 1986. 1,25-Dihydroxycholecalciferol and a human myeloid leukemia cell line (HL-60). *Biochem. J. 204:713.*
30. Kreutz, M., and R. Andressen. 1990. Induction of human monocyte to macrophage maturation in vitro by 1,25-dihydroxyvitamin D_3. *Blood 76:2457.*
31. Walters, M. R. 1992. Newly identified actions of the vitamin D endocrine system. *Endocr. Rev. 13:719.*
32. Xu, H., A. Soruri, R. K. H. Gieseler, and J. H. Peters. 1993. 1,25-Dihydroxyvitamin D_3 exerts opposing effects to IL-4 on MHC class II antigen expression, accessory activity, and phagocytosis of human monocytes. *Scand. J. Immunol. 38:535.*
33. Girasole, G., J. M. Wang, M. Pedrazzoni, G. Poli, C. Balotta, M. Passeri, A. Lazzarin, A. Ridolfo, and A. Mantovani. 1990. Augmentation of monocyte chemotaxis by 1α,25-dihydroxyvitamin D_3: stimulation of defective migration of AIDS patients. *J. Immunol. 145:2459.*
34. Romani, N., S. Gruner, D. Brang, E. Kampgen, A. Lenz, B. Trockenbacher, G. Konwalinka, P. O. Fritsch, R. M. Steinman, and G. Schuler. 1994. Proliferating dendritic cell progenitors in human blood. *J. Exp. Med. 180:83.*
35. Sallusto, F., and A. Lanzavecchia. 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α. *J. Exp. Med. 179:1109.*
36. Chapuis, F., M. Rosenzwajg, M. Yagello, M. Ekman, P. Biberfeld, and J. C. Gluckman. 1997. Differentiation of human dendritic cells from monocytes in vitro. *Eur. J. Immunol. 27:431.*

Downloaded from http://journals.aai.org/jimmunol/article-pdf/164/9/4443/1118540/4443.pdf by guest on 14 May 2025

37. Piemonti, L., S. Bernasconi, W. Luini, Z. Trobonjaca, A. Minty, P. Allavena, and A. Mantovani. 1995. IL-13 supports differentiation of dendritic cells from circulating precursors in concert with GM-CSF. *Eur. Cytokine Network 6:245.*
38. Sallusto, F., M. Cella, C. Danieli, and A. Lanzavecchia. 1995. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. *J. Exp. Med. 182:389.*
39. Grabbe, S., S. Beissert, T. Schwarz, and R. D. Granstein. 1994. Dendritic cells as initiators of immune responses: a possible strategy for tumor immunotherapy? *Immunol. Today* 24:2691.
40. Sallusto, F., and A. Lanzavecchia. 1999. Mobilizing dendritic cells for tolerance, priming, and chronic inflammation. *J. Exp. Med. 189:611.*
41. Schuler, G., and R. M. Steinman. 1997. Dendritic cells as adjuvants for immune-mediated resistance to tumors. *J. Exp. Med. 186:1183.*
42. Morel, A. S., S. Quarantino, D. C. Douek, and M. Londei. 1997. Split activity of interleukin-10 on antigen capture and antigen presentation by human dendritic cells: definition of a maturative step. *Eur. J. Immunol. 27:26.*
43. Buelens, C., V. Verhasselt, D. De Groote, K. Thielemans, M. Goldman, and F. Willems. 1997. Interleukin-10 prevents the generation of dendritic cells from human peripheral blood mononuclear cells cultured with interleukin-4 and granulocyte/macrophage-colony-stimulating factor. *Eur. J. Immunol. 27:756.*
44. Uccini, S., M. C. Sirianni, L.Vincenzi, S. Topino, A. Stopacciaro, I. Lesnoni, C. La Parola, C. Masini, D. Cerimele, M. Cella, et al. 1997. Kaposi's sarcoma cells express the macrophage-associated antigen mannose receptor and develop in peripheral blood cultures of Kaposi's sarcoma patients. *Am. J. Pathol. 150:929.*
45. Biondi, A., T. H. Rossing, J. Bennett, and R. F. Todd. 1984. Surface membrane heterogeneity among human mononuclear phagocytes. *J. Immunol. 132:1237.*
46. Muller, K., and K. Bendtzen. 1996. 1,25- Dihydroxyvitamin D_3 as a natural regulator of human immune function. *J. Invest. Dermatol. 1:68.*
47. Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature 392:245.*
48. Steinman, R. M. 1991. The dendritic cell system and its role in immunogenicity. *Annu. Rev. Immunol. 9:271.*
49. Hart, D. N. J. 1997. Dendritic cells: unique leukocyte populations which control the primary immune response. *Blood 90:3245.*
50. Allavena, P., L. Piemonti, D. Longoni, S. Bernasconi, A. Stopacciaro, L. Ruco, and A. Mantovani. 1998. Interleukin-10 prevents the differentiation of monocytes to dendritic cells but promotes their maturation to macrophages. *Eur. J. Immunol. 28:359.*
51. Piemonti, L., P. Monti, P. Allavena, M. Sironi, L. Soldini, B. E. Leone, C. Socci, and V. Di Carlo. 1999. Glucocorticoids affect human dendritic cell differentiation and maturation. *J. Immunol. 162:6473.*
52. Nakamura, K., T. Takahashi, Y. Sasaki, R. Tsuyuoka, Y. Okuno, M. Kurino, K. Ohmori, S. Iho, and K. Nakao. 1996. 1,25-Dihydroxyvitamin D_3 differentiates normal neutrophilic promyelocytes to monocytes/macrophages in vitro. *Blood 87:2693.*
53. Takahashi, T., K. Nakamura, and S. Iho. 1997. Differentiation of myeloid cells and 1,25-dihydroxyvitamin D_3. *Leukemia Lymphoma 27:25.*
54. Suda, T., T. Shinki, and N. Takahashi. 1990. The role of vitamin D in bone and intestinal cell differentiation. *Annu. Rev. Nutr. 10:195.*
55. Fujikawa, Y., J. M. Quinn, A. Sabokbar, J. O. McGee, and N. A. Athanasou. 1996. The human osteoclast precursor circulates in the monocytes fraction. *Endocrinology 137:4058.*
56. Palucka, K. A., N. Taquet, F. Sanchez-Chapuis, and J. C. Gluckman. 1998. Dendritic cells as the terminal stage of monocyte differentiation. *J. Immunol. 160: 4587.*
57. Cella, M., D. Scheidegger, K. Palmer-Lehmann, P. Lane, A. Lanzavecchia, and G. Albert. 1996. Legation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. *J. Exp. Med. 184:747.*
58. Zhou, L., and T. F. Tedder. 1996. $CD14^+$ blood monocytes can differentiate into functionally mature $CD83^+$ dendritic cells. *Proc. Natl. Acad. Sci. USA 93:2588.*
59. Macatonia, S. E., N. A. Hosken, M. Litton, P. Vieira, C. S. Hsieh, J. A. Culpepper, M. Wysocka, G. Trinchieri, K. M. Murphy, and A. O'Garra. 1995. Dendritic cells produce IL-12 and direct the development of Th1 cells from naive $CD4^+$ T cells. *J. Immunol. 154:5071.*
60. Heufler, C., F. Koch, U. Stanzl, G. Topar, M. Wysocka, G. Trinchieri, A. Enk, R. M. Steinman, and N. Romani. 1996. Interleukin-12 is produced by dendritic cells and mediates T helper 1 development as well as interferon γ production by T helper 1 cells. *Eur. J. Immunol. 26:649.*
61. D'Ambrosio, D., M. Cippitelli, M. G. Cocciolo, D. Mazzeo, P. Di Lucia, L. Rosmarie, F. Sinigaglia, and P. Panina-Bordignon. 1998. Inhibition of IL-12 production by 1,25-dihidroxyvitamin D_3. *J. Clin. Invest. 101:252.*
62. Romani, N., D. Reider, M. Heuer, S. Ebner, E. Kampgen, B. Eibl, D. Niederwieser, and G. Schuler. 1996. Generation of mature dendritic cells from human blood an improved method with special regard to clinical applicability. *J. Immunol. Methods 196:137.*
63. Steinbrink, K., M. Wolfl, H. Jonuleit, J. Knop, and Enk, A. H. 1997. Induction of tolerance by IL-10 treated dendritic cells. *J. Immunol. 159:4772.*
64. Bouillon, R., and H van Baelen. 1981. Transport of vitamin D: significance of free and total concentration of the vitamin D metabolites. *Calcif. Tissue Int. 33:451.*
65. Ross, T. K., H. M. Darwish, and H. F. DeLuca. 1994. Molecular biology of vitamin D action. *Vitam. Horm. 49:281.*
66. Yang, S., C. Smith, J. M. Prahl, and H. F. DeLuca. 1993. Vitamin D deficiency suppresses cell-mediated immunity in vivo. *Arch. Biochem. Biophys. 303:98.*
67. Yang, S., C. Smith, and H. F. DeLuca. 1993. 1α,25-Dihydroxyvitamin D_3 and 19-nor-1α,25-dihydroxyvitamin D_2 suppresses immunoglobulin production and thymic lymphocyte proliferation in vivo. *Biochim. Biophys. Acta 1158:279.*
68. Silva, M. E., M. E. C. Silva, J. R. Nicoli, E. A. Bambirra, and E. C. Vieira. 1993. Vitamin D overload and experimental *Trypanosoma cruzi* infection: parasitological and histopathological aspects. *Comp. Biochem. Physiol. 104:175.*
69. Kolgen, W., H. Van Weelden, S. Den Hengst, K. L. Guikers, R. C. Kiekens, E. F. Knol, C. A. Bruijnzeel-Koomen, W. A. Van Vloten, and F. R. de Gruijl. 1999. $CD11b^+$ cells and ultraviolet-B-resistant $CD1a^+$ cells in skin of patients with polymorphous light eruption. *J. Invest. Dermatol. 113:4.*
70. Dittmar, H. C., J. M. Weiss, C. C. Termeer, R. W. Denfeld, M. B. Wanner, L. Skov, J. N. Barker, E. Schopf, O. Baadsgaard, and J. C. Simon. 1999. In vitro UVA-1 and UVB irradiation differentially perturbs the antigen-presenting function of human epidermal Langerhans cells. *J. Invest. Dermatol. 112:322.*
71. Boonstra, A., and H. F. Savelkoul. 1997. The role of cytokines in ultraviolet-B induced immunosuppression. *Eur. Cytokine Network 8:117.*

Downloaded from http://journals.aai.org/jimmunol/article-pdf/164/9/4443/1118540/4443.pdf by guest on 14 May 2025