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EXPERIMENTAL STUDY

1-α,25-Dihydroxyvitamin D_3 (1,25$(OH)_2D_3$) hampers the maturation of fully active immature dendritic cells from monocytes

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Abstract

Objective: To study the effects of the active metabolite of vitamin D_3, 1,25$(OH)_2D_3$, an immunomodulatory hormone, on the generation of so-called immature dendritic cells (iDCs) generated from monocytes (Mo-iDCs).

Design and methods: Human peripheral blood monocytes were cultured to iDCs in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4 for 1 week, with or without the extra addition of 10^{-8} M 1,25$(OH)_2D_3$ to the culture. Their phenotypes (CD14, CD1a, CD83, HLA-DR, CD80, CD86 and CD40 expression) were examined by fluorescence-activated cell sorting, and their T-cell stimulatory potential was investigated in allogeneic mixed lymphocyte reaction (allo-MLR). Additionally, their *in vitro* production of IL-10, IL-12 and transforming growth factor β (TGF-β) were examined by using the enzyme-linked immunosorbent assay.

Results: When 1,25$(OH)_2D_3$ was added to monocytes in culture with GM-CSF and IL-4, it hampered the maturation of Mo-iDCs. First, the phenotype of the 1,25$(OH)_2D_3$-differentiated DCs was affected, there being impaired downregulation of the monocytic marker CD14 and impaired upregulation of the markers CD1a, CD83, HLA-DR, CD80 and CD40. CD86 was expressed on more 1,25$(OH)_2D_3$-differentiated DCs. Secondly, the T-cell stimulatory capability of 1,25$(OH)_2D_3$-differentiated DCs was upregulated relative to the original monocytes to a lesser degree than DCs differentiated without 1,25$(OH)_2D_3$ when tested in an allo-MLR. With regard to the production of cytokines, *Staphylococcus aureus* cowan 1 strain (SAC)-induced IL-10 production, although not enhanced, remained high in 1,25$(OH)_2D_3$-differentiated DCs, but was strongly downregulated in DCs generated in the absence of 1,25$(OH)_2D_3$. SAC/interferon-γ-induced IL-12 production was clearly upregulated in both types of DC relative to those of the original monocytes, and TGF-β production was downregulated.

Conclusion: Our data confirm earlier reports showing that 1,25$(OH)_2D_3$ hampers the maturation of fully active immunostimulatory major histocompatibility complex (MHC) class II+, CD1a+, CD80+ DCs from monocytes. Our data supplement the data from other reports by showing that the expression of CD86 was upregulated in 1,25$(OH)_2D_3$-differentiated DCs, whilst the capacity for IL-10 production remained high. Collectively, these data are in line with earlier descriptions of suppressive activities of this steroid-like hormone with respect to the stimulation of cell-mediated immunity.

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Introduction

1-α,25-dihydroxyvitamin D_3 (1,25$(OH)_2D_3$) is a steroid hormone known for its ability to regulate calcium metabolism. The presence of the vitamin D_3 receptor in almost all types of immune cells, and the ability of 1,25$(OH)_2D_3$ to affect immune-cell function *in vitro* are indicative of other actions of this hormone. The ability of 1,25$(OH)_2D_3$ to stimulate cell differentiation has been well characterized. For instance, this hormone can inhibit proliferation and induce differentiation of benign cells such as keratinocytes, malignant cells such as prostate, breast and colon adenocarcinoma cells, and various leukemic cells.

1,25$(OH)_2D_3$ also plays a role in the differentiation of benign cells of the myeloid lineage. Many reports have demonstrated that the differentiation of immature monocytes into mature macrophages is fostered by this hormone (1–3). The hormone enhances macrophage-type activities such as phagocytosis and killing of bacteria, adherence, and chemotaxis (4, 5). 1,25$(OH)_2D_3$ is also known for its capacity to induce TGF-β production in monocytes and other cell types (6). Although TGF-β is commonly considered as a tolerance-inducing or immunosuppressive cytokine, it has great plasticity, and its action on immune cells can be inhibitory or stimulatory, depending upon the cell type, the differentiation/activation status, and the environment (7). Thus, its means of regulating immune function is heavily context-dependent.

Data on the effects of $1,25(OH)_2D_3$ on the accessory-cell function of monocytes/macrophages demonstrate reduced antigen-presenting capacity, together with reduced major histocompatibility complex (MHC) class II antigen expression (8, 9). Dendritic cells (DCs) – which are highly specialized antigen-presenting accessory cells (APCs) capable of stimulating naïve T cells – can be generated from monocytes by culture for 1 week under plastic-adherent conditions in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin (IL)-4. The resulting so-called immature dendritic cells (iDCs) express dendritic-type markers such as CD1a, CD83, MHC Class II, CD40, CD80, and CD86, while downregulating the expression of CD14. Their accessory-cell function is also greatly increased. Recent reports indicate that the differentiation of DCs is inhibited by $1,25(OH)_2D_3$. Markers typical of iDCs (CD1a, MHC Class II) are expressed to a lesser degree, and the T-cell stimulatory capacity is reduced (10). However, conflicting data exist regarding the expression of some co-stimulatory molecules. Piemonti (11) found that the expression of CD86 was inhibited in the presence of $1,25(OH)_2D_3$, but that the expression of CD80 was not significantly affected. This is in contrast to data from Berer (12), who found that CD86 expression was unaffected by $1,25(OH)_2D_3$, whereas upregulation of CD80 was prevented. Immature DCs can be further differentiated into mature DCs *in vitro* by culturing them with lipopolysaccharide, TNF-α, IL-1 or CD40-ligand (13, 14). In this mature stage, the capacity for antigen uptake is lost, and the cell specializes in antigen presentation (15, 16). Maturing DCs are also reportedly affected by $1,25(OH)_2D_3$: decreases in IL-12 production and an increase in IL-10 production have been reported (10, 11), but there are, to date, no reports on the effect of $1,25(OH)_2D_3$ on the production of IL-10 and IL-12 in iDCs. The effect of $1,25(OH)_2D_3$ on the production of the immunoregulatory growth factor TGF-β by DCs is also unknown.

In order to further investigate these effects of the immunoregulatory hormone $1,25(OH)_2D_3$ on myeloid DC differentiation, we cultured human peripheral blood monocytes to iDCs in the presence of GM-CSF and IL-4 for 1 week, with the addition of $1,25(OH)_2D_3$ to the culture. We examined the phenotypes (CD14, CD1a, CD83, HLA-DR, CD80, CD86 and CD40 expression) and T-cell stimulatory potential in allogeneic mixed lymphocyte reaction (allo-MLR) of these immature DCs, and investigated their production of IL-10, IL-12 and TGF-β while they were still in their immature state.

Materials and methods

Isolation of monocytes from peripheral blood

Monocytes were isolated from the peripheral blood of healthy blood donors via well-accepted methods. Heparinized blood diluted with an equal volume of phosphate-buffered saline (PBS) was distributed over Ficoll Isopaque (density 1.077 g/ml; Pharmacia, Uppsala, Sweden) and centrifuged for 15 min at 1000 ***g***. Cells collected from the interface and washed were then suspended in RPMI 1640 with 25 mM HEPES and L-glutamine (Biowhittaker, Walkersville, MD, USA), supplemented with 100 U/ml penicillin G, 0.1 mg/ml streptomycin (both from Seromed, Biochrom, Berlin, Germany) and 10% FCS (Biowhitakker) (hereafter known as RPMI 1640^+) added. This cell suspension was distributed over Percoll (density 1.063 g/ml; Pharmacia) then centrifuged for 40 min at 400 ***g***. Cells collected from the interface were washed and suspended in RPMI 1640^+. Monocyte purity was determined by non-specific esterase staining (17). Cell suspensions containing 80% or more monocytes were frozen according to standard procedures and stored in liquid nitrogen, providing a bank for the experiments. Monocytes purified by elutriator centrifugation were also used (courtesy of CLB, Amsterdam, The Netherlands), in order to confirm results obtained via Ficoll/Percoll gradient separation.

Culture of DCs from peripheral blood monocytes

DCs were obtained via the well-established method first described by Sallusto and Lanzavecchia (13). Briefly, monocytes were cultured for 1 week at 37 °C, 5% CO_2 and 100% humidity at a concentration of 3×10^5 cells/ml in RPMI 1640^+ with 800 U/ml GM-CSF and 1000 U/ml IL-4. Feeding of the cultures took place every 2 days, by removing 500 μl culture fluid and replacing this with 1 ml fresh medium with cytokines. In order to test the effects of exposure to $1,25(OH)_2D_3$ on the monocyte-to-DC transition, this hormone was added at an optimal concentration of 10^{-8} M to monocytes in RPMI 1640 culture medium (without FCS) and incubated for 30 min at 37 °C, 5% CO_2 and 100% humidity, after which FCS (10%), GM-CSF (800 U/ml) and IL-4 (1000 U/ml) were added to the culture. $1,25(OH)_2D_3$ was also fed to the cultures every 2 days along with fresh medium and cytokines.

Flow cytometry and (immuno)cytochemistry

For analysis of marker expression by flow cytometry, all cell populations were stained by incubating them for 10 min with mouse anti-human fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated monoclonal antibodies, then washing them three times. The monoclonal antibodies used were My4 (CD14; Beckman Coulter, Hialeah, FL, USA), CD1a (Beckman Coulter), HLA-DR (Becton Dickinson, San Jose, CA, USA) B7·1 (CD80; Becton Dickinson), B7·2 (CD86; PharMingen, Los Angeles, CA, USA), CD83 (Immunotech, Marseilles, France) and CD40 (Serotec, Oxford, Oxon, UK). Immediately after the staining, cells were subjected to fluorescence-activated cell sorting on a FACScan (Becton Dickinson).

Mixed leukocyte reaction (MLR)

Allo-MLRs were performed in order to measure the accessory capacity of the various DC populations generated. Responder T lymphocytes were obtained from healthy donors and isolated using standard procedures with Ficoll-isopaque, Percoll density gradient centrifugation, and nylon wool adherence (Leuko-Pak; Fenwall Laboratories, IL, USA). Most of the non-adhering cells recovered were CD3 positive (>90%). Responder cells (1.5×10^5) were cultured in 96-well, flat-bottom microtitre plates (Nalge Nunc International, Rochester, NY, USA) with different numbers of irradiated (2000 rad) stimulatory cells (monocytes or DCs) to achieve stimulator-to-T-cell ratios of 1:5, 1:10, 1:20 and 1:40. The culture medium used was RPMI 1640 with 25 mM HEPES and L-glutamine, supplemented with 100 U/ml penicillin G, 0.1 mg/ml streptomycin and 10% human A^+ serum, in a total volume of 200 μl per well. The controls used were monocytes or DCs alone, and lymphocytes in the presence of 10–50 μg/ml phytohemagglutinin (Murex Diagnostics Ltd, Dartford, UK). Cultures were performed in triplicate. On day 5, thymidine incorporation was measured by adding 0.5 μCi [^{3}H]thymidine to each well, then harvesting 16 h later. Scintillation was counted on an LKB 1205 Betaplate liquid scintillation counter (Wallac, Turku, Finland).

IL-12, IL-10 and TGF-β production

DCs were placed in 24-well plates (Nalge Nunc International) at a concentration of 5×10^5 cells/ml and cultured for 24 h in RPMI$^+$ containing ultraglutamine (2 mM; Biowhitakker), penicillin/streptomycin (100 U/ml, 100 μg/ml; Biowhitakker) and serum-free medium supplement (SF-1; Corning Costar Europe, Badhoevedorp, The Netherlsands). To stimulate IL-10 production, the culture fluid contained *Staphylococcus aureus* cowan 1 strain (SAC) (1:5000; Calbiochem, La Jolla, CA, USA). The IL-12 production was stimulated by the SAC strain (1:5000) and γ-interferon (IFN-γ, 1000 U/ml; Biomedical Primate Research Centre, Rijswijk, The Netherlands). The production of cytokines was measured by using an enzyme-linked immunosorbent assay (ELISA) as indicated by the manufacturers (IL-10 ELISA Pelikine; CLB; IL-12 Eli-pair; Diaclone, Besançon, France; TGFβ1 ELISA; Biosource International, Nivelles, Belgium).

Statistical analysis

Data were collected in an EXCEL file. Statistical analysis was carried out between sets of measurements ($n => 6$, see the Results section and the table and figure legends for exact details) in the presence or absence of 10^{-8} M $1,25(OH)_2D_3$ by using the parametric *t*-test (two-tailed, unpaired) provided by EXCEL.

Results

$1,25(OH)_2D_3$ influences the morphology and phenotype of DCs

Dose-response curves revealed that an optimal dose of 10^{-8} M $1,25(OH)_2D_3$ reduced the percentage of cells with cellular protrusions (veils and dendrites) from 84±8% (non-vitamin-D-exposed cultures) to 63±9% in the culture to which $1,25(OH)_2D_3$ was added (mean±S.D., $P = 0.002$, $n = 10$).

The phenotype of the DCs in culture was also affected by the addition of $1,25(OH)_2D_3$ (Table 1), resulting in a continued high levels of expression of the monocyte marker CD14 (typically downregulated during culture in GM-CSF and IL-4 alone). The expression of the DC markers CD1a and CD83 (typically upregulated during culture in GM-CSF and IL-4 alone) was still low in DCs differentiated in the presence of $1,25(OH)_2D_3$. The intensity of the expression of the antigen-presenting molecule HLA-DR (typically upregulated during culture in GM-CSF and IL-4 alone) remained low in $1,25(OH)_2D_3$-differentiated cells (Table 1). The addition of $1,25(OH)_2D_3$ to the culture also resulted in significant changes in the expression of the co-stimulatory molecules CD80 (lower expression), CD86 (higher expression) and CD40 (lower expression). In sum, the phenotype of the vitamin-D_3-generated DCs more or less still resembled that of the original monocytes, having persistently high CD14, low CD1a, low CD83, low CD80 and low CD40 expression. The high CD86 expression of vitamin-D_3-generated DCs is remarkable: the percentage of CD86+ DCs remained significantly higher and comparable to that of monocytes, whereas their mean fluorescence intensity was much higher than that of monocytes, which showed only poor expression (Table 1).

Functional differences induced by $1,25(OH)_2D_3$ in DC culture

Dendritic cells were co-cultured with allogeneic T cells in MLRs in order to assess their capacity to stimulate T-cell proliferation. DCs generated under the influence of $1,25(OH)_2D_3$ displayed a greatly reduced ability to stimulate T cells to proliferate (Fig. 1). At a

Table 1 Effect of $1,25(OH)_2D_3$ on the phenotype of monocyte-derived dendritic cells. FACS analysis of iDC populations ($n = 10$ populations for each condition of 10 different donors) generated in the absence or presence of $1,25(OH)_2D_3$; FACS analysis was also performed on the original monocyte populations ($n = 7$). The mean percentage of positive cells (i.e. cells with a signal over that of the IgG control) is given along with the mean fluorescence intensity±standard deviation.

	Percentage of positive cells			Mean fluorescence intensity		
		DCs			DCs	
	Monocytes	−Vitamin D_3	+Vitamin D_3	Monocytes	−Vitamin D_3	+Vitamin D_3
CD14	76±5	14±4*	49±6	2980±712	280±230*	640±41*
CD83	3±2	8±4	4±2	75±40	150±63*	116±38
CD1a	2±2	24±8*	3±1	19±13	270±356*	60±25*,**
HLA-DR	73±6	80±9	68±9	285±97	870±570*	490±350
CD80	5±1	28±6*	15±4	50±34	290±130*	134±102*,**
CD86	75±5	54±5*	64±6**	84±24	400±270*	595±600*
CD40	82±7	94±2	71±12**	84±32	710±297*	320±230*,**

*$P < 0.05$ versus original monocytes; **$P < 0.05$ versus DCs in the absence of vitamin D_3; t-test, EXCEL computer program.

stimulator-to-T-cell ratio of 1:5, the capacity of $1,25(OH)_2$ D_3-generated DCs to stimulate T-cell proliferation was significantly lower than that of the controls. This impairment of stimulatory ability was evident at all ratios tested (P values ranging from $P = 0.01$ to $P = 0.02$, $n = 7–8$). It is also noteworthy that the T-cell-stimulating capacity of the original monocytes was clearly and significantly lower than that of both DC populations.

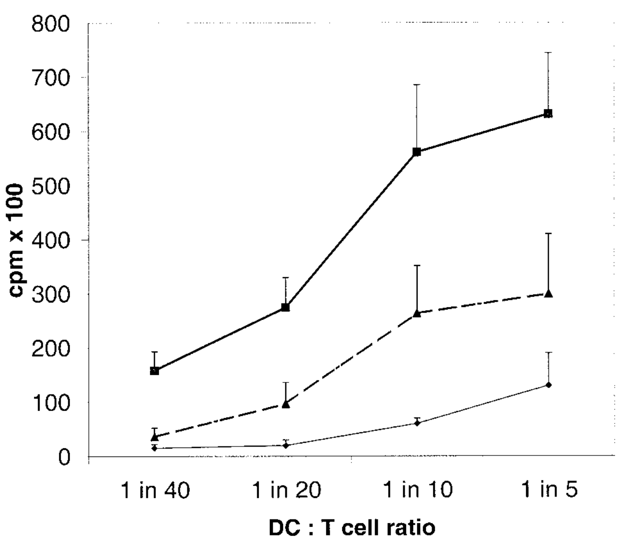

Figure 1 The T-cell-stimulatory capacity in allo-MLR of the original monocytes (◆) and monocyte-derived DCs generated in the absence (■) or presence (▲) of $1,25(OH)_2D_3$. Mean counts per minute (c.p.m.) of [^{3}H]thymidine incorporation are given (±S.D.) for various DC:T-cell ratios in allo-MLR, i.e. 1:40, 1:20, 1:10 and 1:5. Differences between the three conditions are statistically significant at all ratios (P values varying from 0.01 to 0.02, $n = 7–8$ experiments using a different donor in each experiment; t-test, EXCEL computer program).

IL-10, IL12 and TGF-β production

When stimulated for 24 h with SAC strain 1, a well-known inducer of IL-10 production, DCs generated in the presence of $1,25(OH)_2D_3$ produced more of the immunosuppressive cytokine IL-10 than did the control, non-vitamin-D-exposed DCs (1227±221 pg/ml versus 337±83 pg/ml respectively; mean±S.E.M.; $P < 0.01$, $n = 13$ and 9 experiments and donors respectively) (Fig. 2). The IL-10 production of the original monocytes was under such circumstances (1540±534 pg/ml; mean±S.E.M., $n = 12$) (Fig. 2). When SAC strain 1 was not used, IL-10 production was in all instances considerably lower and variable, and therefore was tested in fewer cases. Differences stayed more or less the same, though no longer reaching statistical significance (because of the rather high variability between experiments). The value for

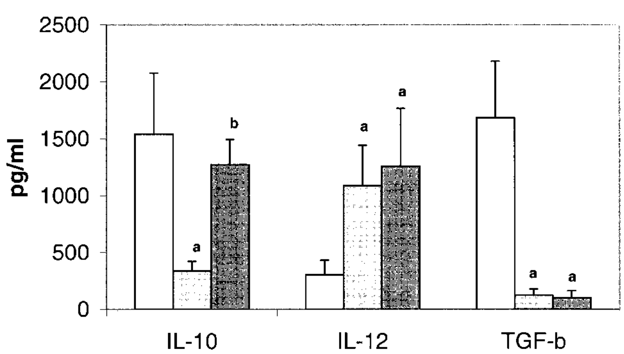

Figure 2 The SAC-stimulated IL-10, the SAC/IFN-γ-stimulated IL-12, and the TGF-β production of original monocytes (white bars) and of monocyte-derived DCs generated in the absence (light-grey bars) or presence (dark-grey bars) of $1,25(OH)_2D_3$. Production rates are given as mean pg/ml/5×10^5 cells (±S.E.M.). a, $P < 0.05$ versus original monocytes; b, $P < 0.05$ versus DCs generated in the absence of $1,25(OH)_2D_3$. For the exact number of experiments, see the text ($n = 9–13$); t-test, EXCEL computer program.

vitamin-D_3-exposed DCs was 94±27 pg/ml (mean± S.E.M., $n = 4$), the value for non-exposed DCs was 26±26 pg/ml ($n = 6$), whereas monocytes produced 174±56 pg/ml ($n = 8$) IL-10.

The presence of $1,25(OH)_2D_3$ in the culture during the differentiation of monocytes into DCs did not significantly affect their capacity to produce IL-12. Under conditions of 24 h stimulation by SAC strain 1 and IFN-γ (both of which are well-known inducers of IL-12 production), 1088±351 pg/ml IL-12 were produced by control DCs; the value was 1056±652 pg/ml for DCs generated under the influence of $1,25(OH)_2D_3$ (mean±S.E.M., $P = 0.31$, $n = 10$ and $n = 7$ respectively) (Fig. 2). The original monocytes produced less IL-12 under these conditions, i.e. 301±129 pg/ml (mean± S.E.M., $n = 17$) (Fig. 2). The omission of SAC strain 1 and IFN-γ from the cultures led to almost no production of IL-12 in all instances (<10 pg/ml).

The TGF-β production of $1,25(OH)_2D_3$-generated and control DCs also did not differ, and was in both instances very low relative to the original monocytes: $1,25(OH)_2D_3$-generated DCs produced 98±65 pg/ml TGF-β (mean±S.E.M.) ($n = 4$) over a 24 h period, control DCs produced 121±57 pg/ml ($n = 4$), and original monocytes produced 1680±500 pg/ml ($n = 7$). SAC strain 1 stimulation during the 24 h production period had no effects.

Discussion

Recent reports indicate a role for $1,25(OH)_2D_3$ in DC development. When monocytes are exposed to $1,25(OH)_2D_3$ during their differentiation into iDCs, the resulting cells are less capable of stimulating T-cell proliferation (10–12). High levels of expression of the monocyte marker CD14 are maintained, whereas CD1a expression is reduced (11, 12). The upregulation of MHC Class II and CD40 is prevented, while conflicting results have been reported regarding the effects of $1,25(OH)_2 D_3$ on the co-stimulatory molecules CD80 and CD86. While Piemonti (11) found that CD86 expression was inhibited and CD80 expression was unaffected by the presence of $1,25(OH)_2D_3$, Berer *et al.* (12) found that CD86 expression was unaffected by $1,25(OH)_2D_3$ and that upregulation of CD80 was prevented. Differences in the endocytic activity of DCs that differentiated in the presence of $1,25(OH)_2D_3$ were also reported by these two groups. There are no reports on the effects of $1,25(OH)_2D_3$ on IL-10 and IL-12 production by immature DCs, but maturing DCs were affected by the presence of $1,25(OH)_2D_3$, as reflected in decreases in the production of the active IL-12 dimer p75 upon exposure to CD40 ligand (10, 11) and an upregulation in IL-10 production (10). This suppressive action of $1,25(OH)_2D_3$ on DC development is in accordance with the numerous reports of a down-regulation of MHC Class II expression in mononuclear cells exposed to this hormone (8, 9). There are no previous reports indicating whether or not this suppressive action of $1,25(OH)_2D_3$ is reflected in the TGF-β production by DCs.

Our data are largely in accordance with this picture of $1,25(OH)_2 D_3$ as an immunosuppressive agent for DC development. We confirmed that $1,25(OH)_2D_3$ hampers the differentiation of monocytes into DCs, generating a population of iDCs with a reduced capacity to induce T-cell proliferation. These iDCs generated while exposed to $1,25(OH)_2D_3$ differed in phenotype from classical iDCs. A significant reduction in the expression of the CD1a and CD40 antigens was seen in the $1,25(OH)_2D_3$-exposed cells. Expression of the co-stimulatory molecule CD80 was inhibited, as has been reported by Piemonti (11), but expression of the co-stimulatory molecule CD86 was increased, in contrast to Piemonti's and Berer's reports of an unchanged and reduced level of expression of this marker in the presence of $1,25(OH)_2D_3$ respectively. A significantly higher level of CD14 expression was seen in the $1,25(OH)_2D_3$-exposed cells, which corresponds with the high level of CD14 antigen expression in mononuclear phagocytes exposed to $1,25(OH)_2D_3$, as noted by numerous investigators. A reduction in CD1a antigen expression was also reported by Dam (18) in experiments applying calcipotriol to normal human skin.

Although $1,25(OH)_2D_3$ has been shown to inhibit IL-12 production by activated macrophages and mature DCs (19), contributing to the immunosuppressive capability of this hormone, we did not find such an effect in iDCs. IL-10 secretion by maturing DCs was recently reported to be enhanced when $1,25(OH)_2D_3$ was added to the culture during lipopolysaccharide-induced maturation (10). In our experiments, we used SAC strain 1 as a stimulus, and also found a high production of IL-10 in $1,25(OH)_2D_3$-differentiated iDCs compared to their original monocyte precursors. In contrast, SAC strain 1-induced IL-10 production by DCs generated without $1,25(OH)_2D_3$ was clearly down-regulated relative to that of the original monocytes. Although it has been demonstrated that calcitriol inhibits epithelial cell growth, possibly by inducing the synthesis of TGF-β (20), and $1,25(OH)_2D_3$ displays actions on cell growth and differentiation that are identical to those exerted by TGF-β, we saw no increase (rather, there was a decrease) in the production of TGF-β by both DC populations in comparison to monocytes. Mercier *et al.* (21) have reported an inhibition in rat liver epithelial cell growth by calcitriol, which was accompanied by a reduction in TGF-β synthesis. In our laboratory, the presence of $1,25(OH)_2D_3$ appeared to have no effect on TGF-β production by immature DCs, providing no evidence for the existence of a paracrine/autocrine loop in the inhibitory effect of $1,25(OH)_2D_3$.

Much *in vitro* investigation has shed light on the presence, and role, of $1,25(OH)_2D_3$ in immune regulation. Monocytes, macrophages and activated lymphocytes all express the vitamin D receptor. $1,25(OH)_2D_3$ is also produced by activated monocytes and macrophages, and has been demonstrated to enhance the antimicrobial function of macrophages (4, 5). Natural killer cell activity is also enhanced by this hormone (5). $1,25(OH)_2D_3$ has also been demonstrated to stimulate T-suppressor-cell function, *in vivo* as well as *in vitro* (22, 23), and is known to inhibit both T- and B-lymphocyte proliferation (24–26) as well as immunoglobulin production (25). $1,25(OH)_2D_3$ also inhibits the production of the growth-promoting lymphokine IL-2 (24, 27, 28), which was discovered to be the mechanism mediating the inhibition of lymphocyte proliferation (26, 29). Activated T lymphocytes can serve as direct targets for $1,25(OH)_2D_3$ (30, 31), but the effects of $1,25(OH)_2D_3$ on these cells are also the result of its actions on monocytes and macrophages in their role as APCs (32). $1,25(OH)_2D_3$ has been demonstrated to both inhibit (27, 33) and promote (28) the IL-1 production of monocytes and macrophages. The production of IL-12, important in the development of T helper-1 based immune responses, has been found to be inhibited by $1,25(OH)_2D_3$ in both monocytes (34) and activated macrophages and mature DCs (19). With regard to *in vivo* effects, immune-modulating properties of $1,25(OH)_2D_3$ and its analogues have been demonstrated in various animal models of autoimmunity. For instance, Mathieu *et al.* (23, 35, 36) showed that $1,25(OH)_2D_3$ and several of its analogues are able to reduce the incidence of type 1 diabetes in the non-obese diabetic (NOD) mouse. Similarly, in the murine model for multiple sclerosis (experimental allergic encephalitis), $1,25(OH)_2D_3$ was shown to prevent the initiation of disease by myelin basic protein (37). Although the use of $1,25(OH)_2D_3$ in organ transplantation has not been very successful in preventing graft rejection (partly because there were complications involving hypercalcemia at the required dosage), various analogues of $1,25(OH)_2D_3$ have been demonstrated to be highly effective in prolonging graft survival without having the hypercalcemic effect of $1,25(OH)_2D_3$ itself (38–40).

In conclusion, exposure to the immunoregulatory hormone $1,25(OH)_2D_3$ during monocyte-to-DC maturation results (in our laboratory) in iDCs with reduced expression of CD1a, CD80 and CD40, reduced ability in the MLR, and a continued high capacity for IL-10 production – all of which are factors likely to account for the immunosuppressive nature of this hormone in T-cell stimulation. Apparently (especially in view of the data previously reported in the literature on the effects of this hormone) $1,25(OH)_2D_3$ skews monocyte differentiation away from APC development and towards the direction of mature phagocytosing macrophages. Thus, this hormone probably favours the development of a strong, non-specific, innate immune reaction over that of an antigen-specific immune response.

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