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Immunology

1α,25-Dihydroxyvitamin D_3 inhibits γ-interferon synthesis by normal human peripheral blood lymphocytes

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ABSTRACT **1α,25-Dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$], the biologically active metabolite of vitamin D_3, inhibited synthesis of γ-interferon (IFN-γ) by phytohemagglutinin-activated peripheral blood lymphocytes (PBLs). A significant reduction of IFN-γ protein levels in PBL culture medium was achieved with a physiologic 1,25-$(OH)_2D_3$ concentration (0.1 nM). 1,25-$(OH)_2D_3$ also inhibited accumulation of IFN-γ mRNA in activated PBLs in a dose-dependent fashion. The ability of 1,25-$(OH)_2D_3$ to modulate IFN-γ protein synthesis was unaltered in the presence of high concentrations of recombinant human interleukin 2. The suppression of IFN-γ synthesis by PBLs was specific for 1,25-$(OH)_2D_3$; the potencies of other vitamin D_3 metabolites were correlated with their affinities for the cellular 1,25-$(OH)_2D_3$ receptor. The time course of 1,25-$(OH)_2D_3$ receptor expression in phytohemagglutinin-activated PBLs was correlated with the time course of 1,25-$(OH)_2D_3$-mediated inhibition of IFN-γ synthesis. In selected experiments, T-lymphocyte-enriched cell preparations were utilized. In these experiments, 1,25-$(OH)_2D_3$ was equally active as in PBL preparations. Finally, we examined the effects of 1,25-$(OH)_2D_3$ on the constitutive IFN-γ production by two human T-lymphocyte lines transformed by human T-lymphotropic virus type I. The cell lines were established from a normal donor (cell line S-LB1) and from a patient with vitamin D-dependent rickets type 2 (cell line Ab-VDR). IFN-γ synthesis by S-LB1 cells was inhibited in a dose-dependent fashion by 1,25-$(OH)_2D_3$, whereas IFN-γ synthesis by Ab-VDR cells was not altered by 1,25-$(OH)_2D_3$. The data presented in this study provide further evidence for a role of 1,25-$(OH)_2D_3$ in immunoregulation.**

The biologically active metabolite of vitamin D_3, 1α,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$], is involved in the regulation of calcium metabolism in humans, by stimulating intestinal calcium absorption and resorption of calcium from bone in a steroid hormone-like fashion (1). Recent evidence indicates that 1,25-$(OH)_2D_3$ can interact with human hemopoietic cells, thus suggesting a wider role for 1,25-$(OH)_2D_3$ in biology than previously was thought. 1,25-$(OH)_2D_3$ promotes differentiation *in vitro* of leukemic (2–4) and normal (5) myeloid precursor cells. Also, peripheral blood lymphocytes (PBLs) have been recognized as target cells for 1,25-$(OH)_2D_3$. Activated, but not resting, lymphocytes express specific 1,25-$(OH)_2D_3$ receptors (6, 7), and 1,25-$(OH)_2D_3$ modulates lymphocyte function by suppressing interleukin 2 (IL-2) secretion and by inhibiting DNA and immunoglobulin synthesis (8–11).

γ-Interferon (IFN-γ) is a lymphokine that plays an important role in modulating cellular immune processes (12). The major source of IFN-γ in humans is the activated T lymphocyte. This study demonstrates that 1,25-$(OH)_2D_3$ sensitively and specifically inhibits IFN-γ production by normal human PBLs, activated *in vitro* by phytohemagglutinin (PHA).

MATERIALS AND METHODS

Cell Cultures. Peripheral blood mononuclear cells, obtained from healthy donors, were isolated by Ficoll-Hypaque (Sigma) density gradients (1.077 g/ml). PBLs were prepared by removing monocytes by their ability to adhere to plastic culture dishes after 6 hr. Wright–Giemsa and nonspecific acid esterase stains of the nonadherent mononuclear cells revealed that <3% were macrophages; the rest had lymphocyte morphology. In selected experiments, a T-lymphocyte-enriched cell population was prepared from PBLs by removing B lymphocytes through adherence to a nylon wool column (13). More than 85% of the nonadherent cells that were eluted from the nylon wool columns were positive for the T-lymphocyte-specific monoclonal antibody anti-Leu-5 (Becton Dickinson). The cells were cultured in 24-well dishes (Corning) at 37°C in humidified air/5% CO_2 in α minimal essential medium (α-MEM; Flow Laboratories) with 10% fetal bovine serum (FBS; Irvine Scientific), penicillin (100 units/ml; Sigma), and streptomycin (100 μg/ml; Sigma). Approximately 10^6 cells in 1 ml of culture medium were used for each point. At the initiation of the culture period, PHA (0.4%; Sigma) and reagents (vitamin D_3 metabolites, IL-2) were added to the cells as indicated. Control cultures received 0.01% ethanol, which was the highest concentration of diluent of 1,25-$(OH)_2D_3$ that was placed in culture. If not indicated otherwise, the cell culture media were harvested after 48 hr. Lymphocytes not exposed to PHA did not produce detectable quantities of IFN-γ. The cell number was determined microscopically, and viability was determined by trypan blue exclusion. By this method, no differences in viability could be detected among cell samples after the various experimental protocols.

The human T-lymphotropic virus type I (HTLV-I)-transformed T-lymphocyte lines S-LB1 and Ab-VDR were maintained in α-MEM/10% FBS/antibiotics at a density of 0.5–1 × 10^6 cells per ml. Cell line Ab-VDR was established from a patient with vitamin D-dependent rickets type 2 (H.P.K., unpublished work). For experiments, the cells were washed in α-MEM and plated into 24-well dishes at identical incubation conditions as described for PBLs. 1,25-$(OH)_2D_3$ and dexamethasone were added at the indicated concentrations for 48 hr. No PHA was used in these experiments.

IFN-γ Measurements. The IFN-γ in culture media was quantitated by a radioimmunoassay (14) (Centocor, Malvern, PA) that employed two murine monoclonal antibodies spe-

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Abbreviations: 25-$(OH)D_3$, 25-hydroxyvitamin D_3; 24*R*,25-$(OH)_2D_3$, 24*R*,25-dihydroxyvitamin D_3; 1,25-$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; 1,24*R*,25-$(OH)_3D_3$, 1α,24*R*,25-trihydroxyvitamin D_3; IFN-γ, γ-interferon; IL-2, interleukin 2; PBL, peripheral blood lymphocyte; HTLV-I, human T-lymphotropic virus type I; PHA, phytohemagglutinin.

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cific for human IFN-γ. In brief, the samples were allowed to bind for 2 hr to the first antibody, which was linked to polystyrene beads. For the next incubation step (2 hr), ^{125}I-labeled second antibody was added to the assay trays. The beads were washed with water and then were assayed for ^{125}I in a gamma counter. The IFN-γ titers are expressed in reference units based on the value of a National Institutes of Health standard. Statistical analyses were performed using Student's *t* test.

RNA Blot Analysis. Lymphocytes were cultured for 48 hr in 75-cm^2 flasks (Corning) under the indicated conditions. Cytoplasmic RNA was isolated by phenol/chloroform extraction (15). The samples (40 μg per lane) were denatured, size-separated by electrophoresis in a 2.2 M formaldehyde/1% agarose gel, and transferred to a nylon membrane filter (ICN). The filter-bound RNA was hybridized with a ^{32}P-labeled (nick-translated) cloned IFN-γ cDNA probe under stringent conditions, washed, dried, and exposed to Kodak XAR-5 film as described (16). Densitometric scanning was performed on an UltroScan XL densitometer (LKB).

1,25-(OH)$_2$D$_3$ Receptor Assay. After 12, 24, 48, and 72 hr in the presence of PHA, the PBLs (pooled from three donors) were harvested, washed three times in phosphate-buffered saline, and homogenized by 10 strokes with a glass/Teflon homogenizer. The receptor levels (25×10^6 cells per time point) were determined as described (17). In brief, the homogenates were incubated for 12 hr with 0.2 pmol of [^{3}H]1,25-(OH)$_2$D$_3$ (100 Ci/mmol; Amersham, Arlington Heights, IL; 1 Ci = 37 GBq) in the absence (triplicates) or presence (duplicates) of a 1000-fold excess of radioinert 1,25-(OH)$_2$D$_3$. Bound [^{3}H]1,25-(OH)$_2$D$_3$ was separated from free [^{3}H]1,25-(OH)$_2$D$_3$ by hydroxylapatite (Bio-Rad). Radioactivity was extracted from hydroxylapatite by 100% ethanol and quantitated by liquid scintillation measurements.

[^{3}H]Thymidine Incorporation. After 48 hr in culture, PBLs were plated in equal volumes (100 μl) into 96-well plates (Corning). Cells were incubated with 1 μCi of [^{3}H]thymidine (6.7 Ci/mmol; New England Nuclear) for 5 hr at 37°C. Cells were harvested onto glass fiber filters with a semiautomatic cell harvester (Skatron, Sterling, VA). The filters were dried, and ^{3}H incorporation was determined by liquid scintillation measurements.

RESULTS

The synthesis of IFN-γ by PBLs was inhibited by 1,25-(OH)$_2$D$_3$ in a dose-dependent fashion. A summary of dose–response studies with PBLs from six different donors is given in Fig. 1*A*. A significant reduction of IFN-γ levels in culture supernatants of PHA-stimulated PBLs was observed at a 1,25-(OH)$_2$D$_3$ concentration as low as 10 pM. In the presence of 0.1 μM 1,25-(OH)$_2$D$_3$, IFN-γ synthesis was decreased 86%. As summarized in Fig. 1*B* and Table 1, treatment of lymphocytes with recombinant human IL-2 resulted in IFN-γ production that averaged 149 ± 58% (mean ± SD) of control values. The relative ability of 1,25-(OH)$_2$D$_3$ to inhibit IFN-γ synthesis by PBLs in the presence of IL-2 was virtually identical to that observed when no IL-2 was added.

Accumulation of IFN-γ-specific mRNA in PHA-activated PBLs after treatment with 1,25-(OH)$_2$D$_3$ at various concentrations was explored by blot hybridization analysis. Incubation of PBLs with 1,25-(OH)$_2$D$_3$ for 48 hr resulted in a dose-dependent decrease of IFN-γ mRNA (Fig. 2). The inhibition of IFN-γ mRNA by 1,25-(OH)$_2$D$_3$ was associated with the inhibition of IFN-γ protein levels.

The potencies of other vitamin D$_3$ metabolites to reduce synthesis of IFN-γ by PBLs were compared to that of 1,25-(OH)$_2$D$_3$ (Fig. 3). Whereas 1,25-(OH)$_2$D$_3$ caused a 50% inhibition of IFN-γ in culture medium at a concentration of

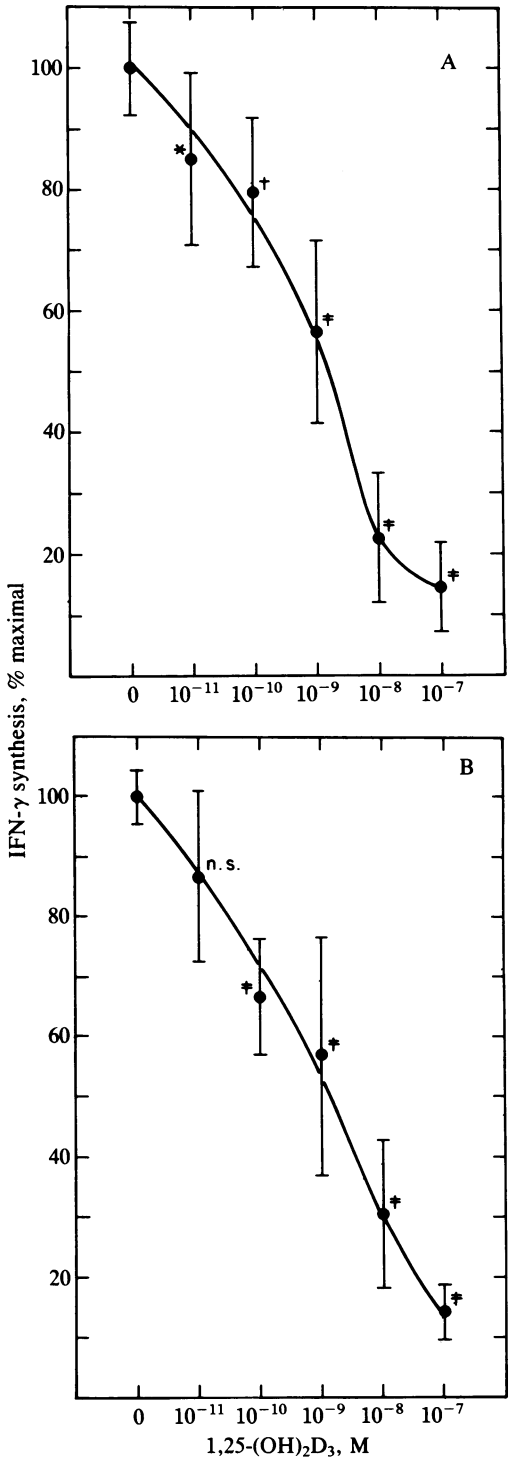

FIG. 1. IFN-γ synthesis by PBLs: 1,25-(OH)$_2$D$_3$ dose–response studies. (*A*) PBLs were cultured for 48 hr in the presence of PHA and various concentrations of 1,25-(OH)$_2$D$_3$. The curve represents the means ± SD of six experiments with PBLs from six normal subjects (duplicate incubations for each point). IFN-γ control values (only PHA) were 311, 362, 184, 204, 143, and 138 units/ml, respectively, for donors 1–6. (*B*) PBLs from three donors (nos. 4 to 6 in *A*) were cultured for 48 hr with PHA, various concentrations of 1,25-(OH)$_2$D$_3$, and 200 ng of recombinant human IL-2 per ml. Data represent the means ± SD of three experiments (duplicate cultures for each point). IFN-γ control values were 271, 192, and 324 units/ml, respectively, for donors 4–6. *, $P < 0.01$; †, $P < 0.002$; ‡, $P < 0.001$; n.s., not significant.

≈2 nM, an ≈10-fold higher concentration of 1α,24*R*,25-trihydroxyvitamin D$_3$ [1,24*R*,25-(OH)$_3$D$_3$] was required for

Table 1. IFN-γ synthesis by PHA-activated lymphocytes treated with 1,25-$(OH)_2D_3$ and IL-2

Treatment		IFN-γ, units/ml per 10^6 cells		
IL-2	1,25-$(OH)_2D_3$	Donor A	Donor B	Donor C
−	−	103 ± 17	172 ± 7	844 ± 84
−	+	11 ± 1	23 ± 4	228 ± 43
+	−	113 ± 6	280 ± 28	994 ± 136
+	+	17 ± 3	42 ± 3	213 ± 72

Freshly isolated PBLs (donors A and B) or T-lymphocyte-enriched cells (donor C) were cultured for 48 hr with PHA (0.4%) plus 1,25-$(OH)_2D_3$ (0.1 μM) and IL-2 (200 ng/ml) as indicated. IFN-γ concentrations in culture supernatants are shown as means ± SD from triplicate cultures.

50% inhibition. 24*R*,25-Dihydroxyvitamin D_3 [24*R*,25-$(OH)_2D_3$] and 25-hydroxyvitamin D_3 [25-$(OH)D_3$] were even less effective, with potencies ≈1/1000th that of 1,25-$(OH)_2D_3$.

The effects of 1,25-$(OH)_2D_3$ (0.1 μM) and IL-2 (200 ng/ml), alone or in combination, on DNA synthesis by PBLs were examined by measuring [^{3}H]thymidine incorporation. The results, obtained after a 48-hr culture period, are shown in Table 2. A moderate decrease of [^{3}H]thymidine incorporation into PBLs (26.7% inhibition, not significant) was noted after 1,25-$(OH)_2D_3$ treatment. IL-2 increased proliferation of PBLs, and 1,25-$(OH)_2D_3$ effected a significant inhibition (39.8%, $P < 0.001$) of this IL-2-dependent increase.

PBLs from two donors and T-lymphocyte-enriched cells from one donor were used to establish a time course of 1,25-$(OH)_2D_3$ (10 nM)-mediated inhibition of IFN-γ synthesis by lymphocytes (Fig. 4*A*). IFN-γ was not detectable in culture medium of resting lymphocytes. Twelve hours after addition of PHA, IFN-γ production by lymphocytes had begun. At this time, 1,25-$(OH)_2D_3$ caused a 55% inhibition of IFN-γ synthesis. After 72 hr, 1,25-$(OH)_2D_3$ reduced IFN-γ production an average of 83%.

The question of whether 1,25-$(OH)_2D_3$ still could inhibit IFN-γ synthesis after prior activation of lymphocytes was examined in another series of experiments. As shown in Fig. 4*B*, a mean 47% decrease of IFN-γ concentrations occurred when 1,25-$(OH)_2D_3$ (10 nM) was added to lymphocytes for the last 24 hr of a 48-hr culture period with PHA. The presence of 1,25-$(OH)_2D_3$ in cultures for the last 6 hr of a 48-hr period resulted in a small reduction (9.8%, not significant) of IFN-γ synthesis (Fig. 4*B*).

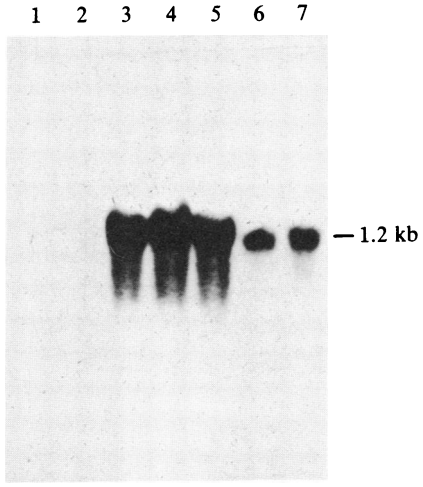

FIG. 2. Blot analysis of IFN-γ mRNA in PHA-stimulated PBLs: 1,25-$(OH)_2D_3$ dose–response study. PBLs were cultured for 48 hr with PHA and various doses of 1,25-$(OH)_2D_3$. Lane 1: human promyelocytic leukemia cells (HL-60). Lane 2: no RNA. Lane 3: PBLs, no 1,25-$(OH)_2D_3$ added. Lane 4: PBLs, 10 pM 1,25-$(OH)_2D_3$, 7% reduction. Lane 5: PBLs, 0.1 nM 1,25-$(OH)_2D_3$, 15% reduction. Lane 6: PBLs, 1 nM 1,25-$(OH)_2D_3$, 57% reduction. Lane 7: PBLs, 10 nM 1,25-$(OH)_2D_3$, 60% reduction. kb, Kilobases.

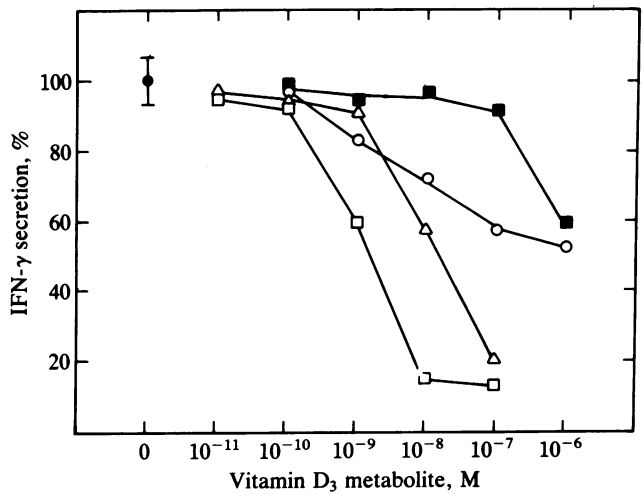

FIG. 3. Effects of various vitamin D_3 metabolites on IFN-γ synthesis. PBLs were cultured for 48 hr with PHA and various concentrations of 1,25$(OH)_2D_3$ (□), 1,24*R*,25-$(OH)_3D_3$ (△), 24*R*,25-$(OH)_2D_3$ (○), or 25-$(OH)D_3$ (■). Means of duplicate incubations are shown. IFN-γ control: 184 units/ml.

We extended our observations by studying two T-lymphocyte lines that had been immortalized by infection with HTLV-I. Both S-LB1 (from a normal donor) and Ab-VDR (from a patient with vitamin D-dependent rickets type 2) constitutively synthesized IFN-γ. S-LB1 cells expressed 1,25-$(OH)_2D_3$ receptors (12 fmol per 10^6 cells), but 1,25-$(OH)_2D_3$ receptors in Ab-VDR cells were virtually undetectable (H.P.K., H.R., J. E. Bishop, and A.W.N., unpublished data). The effects of 1,25-$(OH)_2D_3$ on IFN-γ production by the two cell lines are shown in Fig. 6. 1,25-$(OH)_2D_3$ inhibited IFN-γ synthesis by S-LB1 cells in a dose-dependent manner, although the inhibition was less pronounced than in freshly isolated PHA-activated normal lymphocytes. At comparable concentrations, 1,25-$(OH)_2D_3$ did not reduce IFN-γ production by Ab-VDR cells (Fig. 6). In contrast, treatment of

Table 2. DNA synthesis by PHA-activated PBLs treated with 1,25-$(OH)_2D_3$ and IL-2

Treatment		[^{3}H]Thymidine incorporation,
IL-2	1,25-$(OH)_2D_3$	cpm per 10^6 cells
−	−	11,911 ± 4,446
−	+	8,728 ± 2,097*
+	−	27,007 ± 2,745
+	+	16,280 ± 3,529†

Cells were cultured for 48 hr (10^6 cells per ml) with PHA (0.4%) plus 1,25-$(OH)_2D_3$ (0.1 μM) and IL-2 (200 ng/ml) as indicated. Incorporation of [^{3}H]thymidine was determined as described in *Materials and Methods*. Results represent the means ± SD for nine incubations. Similar results were obtained in another experiment.
*Not significant vs. control [−IL-2, −1,25-$(OH)_2D_3$].
†$P < 0.001$ vs. +IL-2.

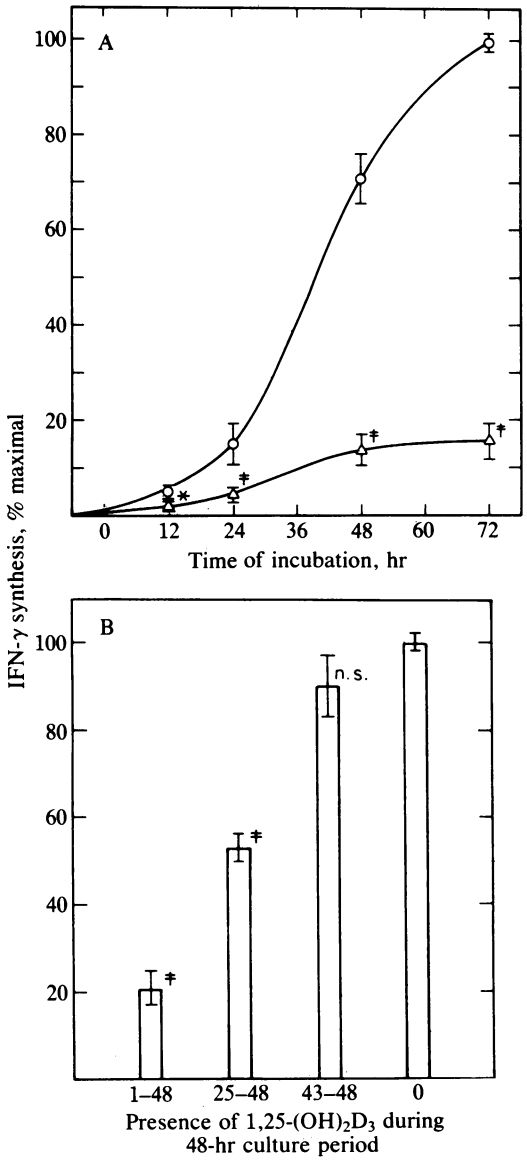

FIG. 4. IFN-γ synthesis by PBLs: Time-course studies. (*A*) Time course of 1,25-$(OH)_2D_3$-mediated inhibition of IFN-γ synthesis. PBLs (donors 1 and 2) and T lymphocytes (donor 3) were incubated with PHA in the absence or presence of 1,25-$(OH)_2D_3$ (10 nM) for the indicated times. Results represent the means ± SD for three experiments (duplicate cultures for each point). Absolute IFN-γ levels after 72 hr were 538 units/ml (donor 1), 218 units/ml (donor 2), and 1340 units/ml (donor 3). (*B*) PBLs from two donors were cultured for 48 hr with PHA; 1,25-$(OH)_2D_3$ (10 nM) was added at various times as indicated. Results represent the means ± SD of two experiments (duplicate cultures). *, $P < 0.05$; ‡, $P < 0.001$; n.s., not significant.

Ab-VDR cells with dexamethasone (10 nM) resulted on average in a 46% inhibition of IFN-γ synthesis.

DISCUSSION

IL-2 has been implicated in the up-regulation of IFN-γ synthesis by T lymphocytes (18, 19), whereas more recent data suggested that the presence of IL-2 protein was not absolutely necessary for transcriptional activation of the IFN-γ gene (20). To rule out the possibility that 1,25-$(OH)_2D_3$-mediated inhibition of IFN-γ synthesis was due to a simultaneous inhibition of IL-2 synthesis by the *seco*-steroid (8, 9), we explored the ability of 1,25-$(OH)_2D_3$ to modulate IFN-γ synthesis in the presence of high concentrations (200 ng/ml) of recombinant human IL-2. As observed previously, IL-2 increased IFN-γ quantities in lymphocyte cultures (18, 19). However, the IL-2 did not markedly influence the relative inhibitory effects of 1,25-$(OH)_2D_3$ on IFN-γ synthesis (Fig. 1*B* and Table 1), suggesting that this effect of the hormone is mostly independent of IL-2. Another observation supporting this conclusion was made in S-LB1 cells. This HTLV-I-transformed T-lymphocyte line constitutively synthesized IFN-γ, and 1,25-$(OH)_2D_3$ produced a dose-dependent inhibition of IFN-γ synthesis (Fig. 6). However, S-LB1 cells did not express IL-2 mRNA as determined by blot hybridization analysis (A.T. and H.P.K., unpublished data). Therefore, the inhibition of IFN-γ synthesis in these cells by 1,25-$(OH)_2D_3$ appears to be independent of IL-2.

The effects of 1,25-$(OH)_2D_3$ on IFN-γ production could have been associated with an inhibition of lymphocyte proliferation by the hormone. In the presence of 1,25-$(OH)_2D_3$ (0.1 μM), DNA synthesis by PBLs after 48 hr was slightly inhibited (Table 2), whereas the same concentration of 1,25-$(OH)_2D_3$ caused a >3-fold greater reduction of IFN-γ

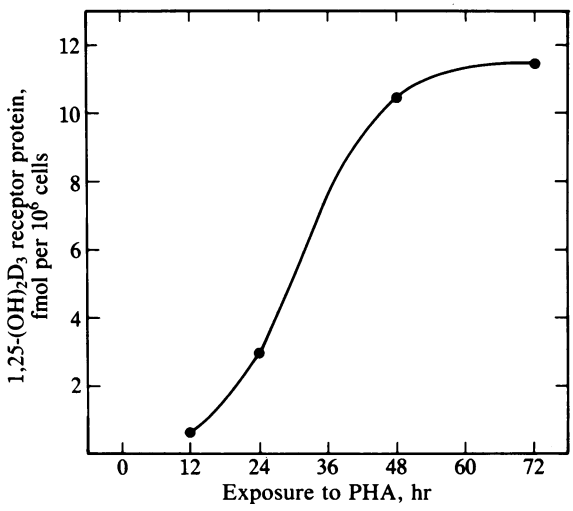

FIG. 5. Time course of 1,25-$(OH)_2D_3$-receptor expression in PHA-stimulated PBL.

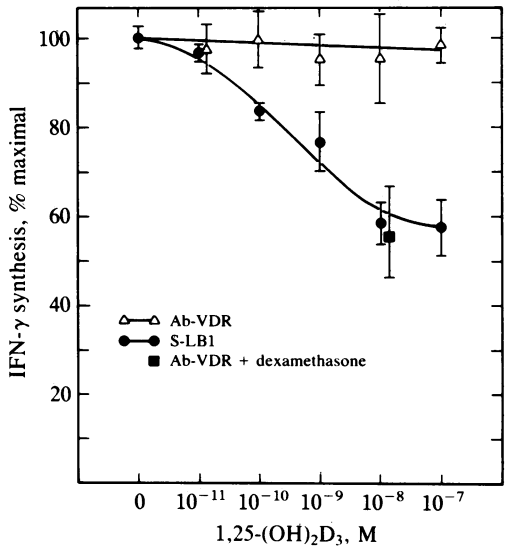

FIG. 6. IFN-γ synthesis by HTLV-I-transformed human T-lymphocyte cell lines: Effects of 1,25-$(OH)_2D_3$. Results are the means ± SD of three (Ab-VDR) or two (S-LB1) experiments (duplicate cultures for each point). Production of IFN-γ after 48 hr was 546 ± 19 units/ml for Ab-VDR and 103 ± 5 units/ml for S-LB1.

synthesis by PBLs (Fig. 1*A*), suggesting that the effect of $1,25\text{-}(OH)_2D_3$ on IFN-γ production is not associated with nonspecific inhibition of DNA synthesis. Rigby *et al.* (9) and Lemire *et al.* (21) reported that $1,25\text{-}(OH)_2D_3$ inhibited lymphocyte proliferation with much higher potency than noted here. However, in their studies DNA synthesis was measured at $\geq$72 hr after activation of cells.

Bhalla *et al.* (10) studied proliferation of murine T-cell clones and suggested that $1,25\text{-}(OH)_2D_3$ was involved in some early events of T-cell activation. In terms of IFN-γ synthesis (as measured at 48 hr after activation), $1,25\text{-}(OH)_2D_3$ clearly was effective when added 24 hr after PHA (Fig. 4*B*). These results show that $1,25\text{-}(OH)_2D_3$ does not necessarily have to be present at the initiation of lymphocyte activation in order to reduce IFN-γ production.

Results from several experiments clearly suggested a receptor-mediated effect of $1,25\text{-}(OH)_2D_3$ on IFN-γ synthesis. Inhibition of IFN-γ production was specific for $1,25\text{-}(OH)_2D_3$ (Fig. 3). The potencies of other vitamin D_3 metabolites to inhibit IFN-γ production were correlated with their known affinities for the cellular $1,25\text{-}(OH)_2D_3$ receptor. Further, the time course of $1,25\text{-}(OH)_2D_3$ receptor expression in activated PBLs (Fig. 5) was correlated with the time-dependent effects of $1,25\text{-}(OH)_2D_3$ on IFN-γ synthesis (Fig. 4*A*). Probably the strongest evidence for a receptor-mediated mechanism was obtained by studying the HTLV-I-transformed T-lymphocyte line Ab-VDR, established from a patient with vitamin D-dependent rickets type 2. Such patients have, to a varying degree, functionally defective receptors for $1,25\text{-}(OH)_2D_3$, resulting in resistance to the actions of the *seco*-steroid. Cellular receptors for $1,25\text{-}(OH)_2D_3$ in Ab-VDR cells were undetectable. Synthesis of IFN-γ by Ab-VDR cells was not inhibited by $1,25\text{-}(OH)_2D_3$. In contrast, S-LB1 T cells, established from a normal donor, were sensitive to $1,25\text{-}(OH)_2D_3$ (Fig. 6). Moreover, dexamethasone decreased IFN-γ synthesis by Ab-VDR cells (Fig. 6), demonstrating a resistance specific for $1,25\text{-}(OH)_2D_3$.

Lemire *et al.* (11) identified helper/inducer T cells as targets for $1,25\text{-}(OH)_2D_3$ in terms of modulation of immunoglobulin synthesis. In contrast, Manolagas *et al.* (22) reported that $1,25\text{-}(OH)_2D_3$-mediated inhibition of lymphocyte proliferation and IL-2 synthesis was dependent on the presence of monocytes in cell cultures. Our results with the T-lymphocyte line S-LB1 suggested that IFN-γ production was modulated by $1,25\text{-}(OH)_2D_3$ by a direct effect on T lymphocytes. This hypothesis was supported by results from selected experiments in which highly enriched T-lymphocyte preparations were employed. As expected, overall IFN-γ concentrations were markedly higher in T-lymphocyte cultures than in PBL cultures. No differences in the efficacy of $1,25\text{-}(OH)_2D_3$ in inhibiting IFN-γ synthesis were observed between the two types of cultures. However, since completely pure T-lymphocyte preparations were not used, our results do not exclude the possibility that the presence of residual accessory cells (B lymphocytes, monocytes) is a prerequisite for $1,25\text{-}(OH)_2D_3$ to affect IFN-γ synthesis. Further studies with subpopulations of normal lymphocytes will be necessary to clarify this question.

In recent studies in our laboratories, we found that cultured normal human bone marrow and pulmonary macrophages, when exposed to recombinant IFN-γ, synthesized $1,25\text{-}(OH)_2D_3$. The structure of $1,25\text{-}(OH)_2D_3$ was determined in both cases by mass spectrometry (H.R., H.P.K., and A.W.N., unpublished results). Our results suggest that macrophages and lymphocytes, when activated during an immune response, can interact via IFN-γ and $1,25\text{-}(OH)_2D_3$ in a paracrine fashion. These findings provide further evidence for a role of $1,25\text{-}(OH)_2D_3$ in immunoregulation.

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1. Norman, A. W., Roth, J. & Orci, L. (1982) *Endocrine Rev.* **3,** 331–366.
2. Murao, S., Gemmell, M. A., Callahan, M. F., Anderson, N. L. & Huberman, E. (1983) *Cancer Res.* **43,** 4989–4996.
3. Bar-Shavit, Z., Teitelbaum, S. L., Reitsma, P., Hall, A., Pegg, L. E., Trial, J. & Kahn, A. J. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 5907–5911.
4. Mangelsdorf, D. J., Koeffler, H. P., Donaldson, C. A., Pike, J. W. & Haussler, M. R. (1984) *J. Cell Biol.* **98,** 391–398.
5. Koeffler, H. P., Armatruda, T., Ikekawa, N., Kobayashi, Y. & DeLuca, H. F. (1984) *Cancer Res.* **44,** 5624–5628.
6. Provvedini, D. M., Tsoukas, C. D., Deftos, L. J. & Manolagas, S. C. (1983) *Science* **221,** 1181–1183.
7. Bhalla, A. K., Amento, E. P., Clemens, T. L., Holick, M. F. & Krane, S. M. (1983) *J. Clin. Endocrinol. Metab.* **57,** 1308–1310.
8. Tsoukas, C. D., Provvedini, D. M. & Manolagas, S. C. (1984) *Science* **224,** 1438–1440.
9. Rigby, J. W. C., Stacy, T. & Fanger, M. W. (1984) *J. Clin. Invest.* **74,** 1451–1455.
10. Bhalla, A. K., Amento, E. P., Serog, B. & Glimcher, L. H. (1984) *J. Immunol.* **133,** 1748–1754.
11. Lemire, J. M., Adams, J. S., Kermani-Arab, V., Bakke, A. C., Sakai, R. & Jordan, S. C. (1985) *J. Immunol.* **134,** 3032–3035.
12. Trinchieri, G. & Perussia, B. (1985) *Immunol. Today* **6,** 131–136.
13. Henry, C. (1980) in *Selected Methods in Cellular Immunology,* eds. Mishel, B. B. & Shigi, S. M. (Freeman, San Francisco), pp. 211–221.
14. Chang, T. W., McKinney, S., Liu, V., Kung, P. C., Vilcek, J. & Le, J. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 5219–5222.
15. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
16. Goldberg, D. A. (1980) *Proc. Natl. Acad. Sci. USA* **81,** 5794–5798.
17. Wecksler, W. R. & Norman, A. W. (1979) *Anal. Biochem.* **92,** 314–323.
18. Reem, G. H. & Yeh, N. H. (1984) *Science* **225,** 429–430.
19. Vilcek, J., Henriksen-DeStefano, D., Siegel, D., Klion, A., Robb, R. J. & Le, J. (1985) *J. Immunol.* **135,** 1851–1856.
20. Kroenke, M., Leonard, W. J., Depper, J. M. & Greene, W. C. (1985) *J. Exp. Med.* **161,** 1593–1598.
21. Lemire, J. M., Adams, J. S., Sakai, R. & Jordan, J. C. (1984) *J. Clin. Invest.* **76,** 657–661.
22. Manolagas, S., Tsoukas, C., Provvedini, D. & Landgraf, B. (1986) *J. Bone Min. Res.* **1,** Suppl. 1, 12 (abstr.).