1,25-Dihydroxyvitamin D₃ Modulates Bone Marrow Macrophage Precursor Proliferation and Differentiation

UP-REGULATION OF THE MANNOSE RECEPTOR

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1,25-Dihydroxyvitamin D₃ (1,25-(OH)₂D₃), the biologically active form of vitamin D₃, has been shown to inhibit proliferation and promote monocyte differentiation of leukemic cell lines. In the present communication, we extend these observations to normal bone marrow macrophage precursors, and 1) identify the stage of monocytic maturation wherein the steroid exerts its antiproliferative effect, and 2) demonstrate that 1,25-(OH)₂D₃ promotes bone marrow macrophage differentiation as manifest by specific up-regulation of the lineage-specific membrane protein, the mannose-fucose receptor.

In these experiments, the 1,25-(OH)₂D₃-mediated inhibitory effect on colony formation was shown to be independent of attendant levels of colony stimulating factor-1 and targeted through the adherent bone marrow macrophage precursor. Examination of this steroid-sensitive adherent precursor population demonstrates that its specific binding of ¹²⁵I-mannose bovine serum albumin spontaneously and progressively increases with time in culture. Whereas adherent bone marrow macrophages cultured for 2 days express 3 × 10⁴ mannose receptors/cell, the number of binding sites increases to 7 × 10⁴/cell by day 4.

When bone marrow macrophage precursors are exposed to 1,25-(OH)₂D₃, an additional stepwise enhancement of ¹²⁵I-mannose bovine serum albumin obtains with time. Four days of culture with the steroid results in 1.6 × 10⁴ mannose receptors/cell, a 100% increase as compared to control cells. Neither duration of culture nor exposure to 1,25-(OH)₂D₃ alters the Kd of ¹²⁵I-mannose bovine serum albumin which approximates 3–5 × 10⁻⁹ M⁻¹. Finally, the “specificity” of vitamin D-mediated up-regulation of the mannose receptor was established by demonstrating that the steroid does not alter binding of ¹²⁵I-α-thrombin by bone marrow-derived macrophage precursors.

1,25-Dihydroxyvitamin D₃ (1,25-(OH)₂D₃),¹ the most potent metabolite of calciferol, is classically viewed as a natural compound pivotal to calcium homeostasis. It has recently been established, however, that the distribution of 1,25-(OH)₂D₃ receptors is virtually ubiquitous, and in fact, the biological functions of this agent go well beyond the regulation of blood calcium levels (1, 2). One such function is the steroid’s ability to promote “differentiation” of a variety of transformed cells (3–9).

HL-60s are a human promyelocytic leukemia line with the capacity to differentiate along either the myeloid or monocytic pathway, and we demonstrated earlier that 1,25-(OH)₂D₃ prompts these cells to mature along a monocytic pathway (5). This vitamin D metabolite-induced differentiation is characterized by both decreased cellular proliferation and induction of a variety of mononuclear phagocyte-specific surface antigens. Other investigators have confirmed these results and report similar findings using different human (U937) and murine (M-1) cell lines (3, 7, 8).

While these studies of transformed lines are interesting, there is only suggestive evidence that 1,25-(OH)₂D₃ may have similar effects on authentic bone marrow-derived mononuclear phagocytes (BMDM) (10, 11). These data come from several laboratories wherein freshly harvested bone marrow cells were treated with vitamin D metabolites leading to inhibition of mononuclear phagocyte colony formation. While such information raises the possibility that vitamin D directly induces monocytic differentiation of marrow residing colony forming units (CFU-c(s)), several additional criteria must be fulfilled to confirm this hypothesis. First, since bone marrow contains a wide variety of cell types, the precise target cell(s) through which 1,25-(OH)₂D₃ exerts its inhibition of mononuclear phagocyte colony formation is unknown. Hence, any claim that the steroid directly affects monocyte precursors must entail isolation of the target cell and demonstration that it is of mononuclear phagocyte origin. If the target cell is of monocytic origin, then a mononuclear phagocyte-specific marker which increases as authentic BMDMs develop in culture must be identified and used to characterize in vitro monocytic differentiation. Finally, if 1,25-(OH)₂D₃ does promote monocytic differentiation in authentic BMDMs, it should enhance the expression of this marker and not universally influence all cell membrane receptors.

We demonstrate herein that 1,25-(OH)₂D₃ inhibits proliferation and promotes monocytic differentiation of authentic

¹ The abbreviations used are: 1,25-(OH)₂D₃, 1,25-dihydroxyvitamin D₃; ¹²⁵I-Man-BSA, ¹²⁵I-mannosylated bovine serum albumin; BMDM, bone marrow-derived mononuclear phagocytes; CFU-c(s), marrow residing colony forming units(s); CSF-1, colony stimulating factor-1; NA, nonadherent; Adh, adherent; MEM, minimal Eagle’s medium.
BMDMs. In our evaluation of 1,25(OH)2D3's influence on authentic monocytopoiesis, we 1) isolate the adherent mononuclear phagocyte colony forming precursor as the target cell through which vitamin D inhibits colony formation; 2) identify and characterize the mannose receptor as a specific, qualitative marker of BMDM differenntiation; and 3) demonstrate that 1,25(OH)2D3 specifically promotes expression of this differentiation-associated cell membrane receptor.

**EXPERIMENTAL PROCEDURES**

**Materials**—Vitamin D metabolites were provided by Dr. Milan Ushakov (Hoffman-LaRoche). Unless otherwise specified, all chemicals were obtained from Sigma. Horse and fetal calf serum were purchased from Hazelton Durlach Research Products (Denver, PA). Mannose-BSA (42 mol of sugar/mol of protein) was purchased from E-Y Laboratories (San Mateo, CA).

**Preparation of Stage I CSF-1**—Stage I CSF-1 was prepared by modification of the method of Stanley (12). Serum-free conditioned media from L929 cells was first chromatographed on a batch calcium phosphate method. Specifically, 250 ml of calcium phosphate gel (300 ml of 0.4 M Na2HPO4 plus 3 liters of 0.057 M CaCl2) was added per liter of L929 conditioned media and stirred overnight. The supernatant was centrifuged and the gel rinsed twice with 5 ml of induction (6.5) and once with 94 ml Na2HPO4. Supernatants were collected from the high phosphate rinses (6) and dialyzed against deionized water.

**CSF-1 levels** were then determined via radioimmunoassay by E. R. Stanley (Albert Einstein Medical School, Bronx, NY). Lowry assay (15) was used for protein determination. Final specific activities were typically 106 units/mg of protein.

**Complete Culture Medium**—a modification of Eagle's medium (MEM) (Sybrwon, Washington, D. C.) plus 15% fetal calf serum plus penicillin (100 units/ml) and streptomycin (100 µg/ml).

**Nonadherent Marrow Cells**—Nonadherent marrow cells were obtained from cultures of the bone marrow of 6-12-week-old male A/J mice (Jackson Laboratories, Benton Harbor, MI) and prepared as described previously (14). The ends of freshly harvested femurs were excised, and the marrow cells were collected by flushing the medullary cavity with ice-cold a-MEM through a 25-gauge needle. The marrow plug was dispersed by several passages through an 18-gauge needle, and the cells were pelleted (800 x g for 7 min at 4 °C). The pellet was resuspended in ice-cold a-MEM and the number of nucleated cells determined by counting an aliquot of the resuspended cells in 2% acetic acid. Cells (1 x 106 cells/ml) were then seeded into tissue culture dishes (Costar, Cambridge, MA) at a density of 3.4 x 106 cells/cm2 in the presence of complete media containing 900 units/ml of Stage I CSF-1. After a 24-h incubation, nonadherent cells were collected and the adherent cells discarded.

**Adherent Cells**—Adherent BMDM were washed with cold phosphate-buffered saline, detached from the tissue culture plastic after a 5-min incubation at 20 °C in 0.005% Trypsin (Behring Diagnostics), and counted by hemocytometer.

**Man-BSA Iodination** (15)—100 µg of mannose-BSA was mixed with 1 Ci of Na125I (Amersham Corp.) and 300 µg of chloramine T in 80 µl of 0.1 M Na2HPO4 buffer (pH 7.6). The reaction was terminated after 10 min on ice by addition of 190 µl of sodium metabisulfite (2.4 mg/ml) and 190 µl of potassium iodine (10 µg/ml). The sample was then precipitated with trichloroacetic acid (20%), and the sediments were washed with cold ethanol and dried. The iodination procedure was confirmed by gamma counting. Protein determination was performed by the Miller method (16), and specific activity was typically 5-10 x 106 cpm/µg of Man-BSA with >95% of total counts trichloroacetic acid-precipitable. Ligand was used within 2 weeks of iodination.

**a-Thrombin Binding Assay**—The preparation and characterization of a-thrombin have been described previously (18). 100 µg of a-thrombin were mixed with 1 mCi of Na125I (Amersham Corp.) in a solution of 2.5 mg of benzamidine hydrochloride/100 ml of 0.1 M NaCl plus 30 mEq of sodium phosphate (pH 7.0). After 15 min on ice, the sample was applied as a P-2 column (Bio-Rad) buffered with 0.4% BSA in phosphate-buffered saline (pH 6.8). 1.0-ml samples were collected and active fractions identified by gamma counting. Typical specific activities were 5-10 x 106 cpm/µg of a-thrombin with >95% total counts trichloroacetic acid-precipitable. Ligand was used within 2 weeks of iodination.

**125I-Man-BSA Binding Assay**—Binding determinations at 4 °C of 125I-Man-BSA to adherent BMDM involved slight modifications of techniques previously described (15). Such procedures resulted in 10-20% of total cell-associated counts being nonspecific binding. All steps in 125I-Man-BSA binding studies were performed at 4 °C. To prepare cells for these studies, BMDM were washed three times (0.4 ml/well/wash) with HHBG (Hanks' balanced salt solution, 10 mM Hepes, 10 mM Tris, 0.1% glucose, and 10 mg/ml BSA (pH 7.1)) and incubated with 0.2 ml of various concentrations of 125I-Man-BSA (in HHBG plus 0.2 ml of HHBG 2 x 106 cells/ml) for 48 h. After 48 h, incubation medium was removed by aspiration, and cell layers were quickly rinsed six times with Hanks' balanced salt solution. Cells were dissolved in 1.0 n NaOH (0.5 ml/well) and cell-bound radioactivity was measured by gamma counting. Duplicate values were determined for all binding points. Transformation of binding data to determine dissociation constants and to estimate the number of available binding sites was performed by methods of Scatchard (19).

**a-Thrombin Binding Assay**—All a-thrombin binding studies were performed at 4 °C. BMDM were placed on ice and rinsed three times with HHBG (0.4 ml/rinse). All wells received various amounts of unlabeled a-thrombin (2-150 µg/ml), 0.2 ml of HHBG, plus 0.2 ml of 0.5 µg/ml 125I-a-thrombin (100 cpm/mg of protein). Equilibrium binding was achieved after a 24-h incubation in both control and steroid-treated cells. After this 24-h incubation, cell-associated 125I-a-thrombin was determined by rinsing, harvesting, and counting techniques as described for 125I-mannose BSA determinations.

When competition binding data of this type are plotted as 125I-a-thrombin bound versus dose of unlabeled a-thrombin added, the then run on an x/y graph and asymptote approaches a horizontal line (see Fig. 7A). The horizontal asymptote of this curve represents nonspecific binding. The most accurate method for predicting the asymptote to such a curve employs a computer-associated mathematical analysis (20, 21). To estimate nonspecific binding, we used a 'lignand competition' computer program designed specifically for analysis of data from ligand-binding experiments. The data-fitting portion of this program 'best fit' our binding data to a simple one-site binding model, and then generated a statis-
1,25-Dihydroxyvitamin D₃ Modulates Macrophage Differentiation

RESULTS

1) Obtaining Homogeneous Populations of Mononuclear Phagocyte Colony Forming Precursors—Formation of CFU-c(s) from bone marrow cells in tissue culture requires a source of colony stimulating factor (CSF) (22). In addition to CSF-responsive cells, freshly harvested bone marrow also contains stromal (fibroblast-like) cells which will proliferate independently of CSF (14). Hence, freshly harvested marrow cells cultured in the presence of nonspecific (i.e. multiple) CSFs will produce a variety of hematopoietic as well as nonhematopoietic cells. To obtain a homogeneous population of bone marrow-derived mononuclear phagocyte colonies, two conditions must therefore be satisfied. Hematopoietic precursor cells must be 1) separated from the “contaminating” stromal cells as well as 2) exposed to the mononuclear phagocytic specific colony stimulating factor (CSF-1).

We satisfied these two conditions by employing the method of Tushinski et al. (14) to separate the mononuclear phagocyte precursors from contaminating stromal cells, and by culturing the nonstromal cell population with purified CSF-1. Based on the facts that 1) greater than 99% of 7-day colonies are positive for monocyte-specific α-naphthyl acetate esterase activity, and 2) more than 99% of the cells die within 72 h of removal of CSF-1, the lineage commitment of our colony forming cell population is exclusively monocytic.

2) Effect of Vitamin D on Mononuclear Phagocyte Colony Formation—With the capacity to culture homogeneous populations of mononuclear phagocytes in hand, we examined the effect of vitamin D on the formation of CFU-c(s), with the aim of determining if 1,25-(OH)₂D₃ mediates its colony-inhibiting influence through mononuclear phagocyte precursors. Stromal cell-free marrow cell populations were plated into 7-day colony forming assays in the presence of increasing doses of 1,25-(OH)₂D₃. The effect of these steroids on mononuclear phagocyte colony formation is shown in Fig. 1. Vitamin D inhibits colony formation in a dose-dependent, metabolite-specific fashion. The ID₅₀ values of 1,25-(OH)₂D₃ and 25-hydroxyvitamin D₃ are 10⁻⁴ and 10⁻⁵ M, respectively. This two log order difference in the potencies of these metabolites reflects their relative affinities for the vitamin D receptor, and it suggests that these steroidal effects are receptor-mediated.

3) Effect of CSF-1 on 1,25-(OH)₂D₃’s Inhibition of Mononuclear Phagocyte Colony Formation—Since CSF-1 is directly responsible for the survival, proliferation, and differentiation of BMDMs, we next sought to determine if vitamin D’s influence on mononuclear phagocyte colony formation is regulated by the concentration of CSF-1 to which they are exposed. Seven-day colony forming assays were performed in either the presence (50 nm) or absence of 1,25-(OH)₂D₃, with doses of CSF-1 ranging from 50 to 1000 units/ml. The influence of CSF-1 on 1,25-(OH)₂D₃-mediated inhibition of colony formation is shown in Fig. 2. It can be seen that 1,25-(OH)₂D₃’s colony inhibiting effect is present throughout the CSF-1 dose-response curve and that the degree of inhibition by 1,25-(OH)₂D₃ (~50%) is independent of the level of CSF-1 employed. These data demonstrate that 1,25-(OH)₂D₃ inhibits mononuclear phagocyte colony formation at all levels of the mitogen.

4) Establishing Time Course of Colony Development—Production of CFU-c(s) from bone marrow cells in culture is a dynamic process. In simplified terms, the development of such units involves: 1) marrow cell production of nonadherent (NA) CFU-c(s) (Diagram 1A), 2) adherence (Adh) of the NA-CFU-c (Diagram 1B), and 3) proliferation of the Adh-CFU-c (Diagram 1C) (23). In this next series of experiments, we sought to determine the time course of each of these developmental steps, with the aim of understanding how each contributes to mononuclear phagocyte colony formation.

We first examined the length of time in culture that bone marrow cells would continue to produce NA-CFU-c(s) (Diagram 1A). This was accomplished by plating marrow cells in culture and, at 24-h intervals (for 4 days), by reculturing a portion of the remaining NA cell fraction to assess its colony forming capacity. It was found that, for at least 4 days, marrow cells produce NA-CFU-c(s) at a constant rate (data not shown).

We next studied the rate of adherence to plastic of the NA-CFU-c (Diagram 1B). First, marrow cells were plated into the 7-day colony forming assay. Then, at designated intervals postplating (6 h to 4 days), the NA fraction was removed and
Colony formation from bone marrow cells involves: A, marrow cell production of nonadherent CFU-c(s); B, adherence of the nonadherent CFU-c(s); and C, proliferation of the adherent CFU to form an adherent colony.

**Diagram 1. Schematic representation of mononuclear phagocyte colony formation.** Formation of adherent monocyte colony from bone marrow cells involves: A, marrow cell production of nonadherent CFU-c(s); B, adherence of the nonadherent CFU-c(s); and C, proliferation of the adherent CFU to form an adherent colony.

1. **Effect of time in culture on adherence of CFU-c(s).** Marrow cells were placed in the colony forming assay and at designated intervals postplating (6 h to 4 days), the nonadherent marrow fraction was removed, and the adherent cells were cultured for the remainder of the 7-day assay. All values shown are the means of quadruplicate determinations ± S.E.

2. **Diagram 1A.** Schematic representation of mononuclear phagocyte colony formation. Formation of adherent monocyte colony from bone marrow cells involves: A, marrow cell production of nonadherent CFU-c(s); B, adherence of the nonadherent CFU-c(s); and C, proliferation of the adherent CFU to form an adherent colony.

3. **Effect of 1,25-(OH)₂D₃ on adherent versus nonadherent CFU-c(s).** Marrow cells were placed in the colony forming assay in the presence (C, D) or absence (A, B) of 1,25-(OH)₂D₃ (50 nm). After 6 h in culture (Nonadherent Phase), the nonadherent cell fraction was removed, and the remaining adherent cell fraction from both groups (A, B) was cultured in control (A, C) or 1,25-(OH)₂D₃-containing medium (B, D). All values shown are the means of quadruplicate determinations ± S.E.
CFU-c formation exclusively via the Adh-CFU-c(s) (Diagram 1C) and provide additional evidence that the steroid influences neither the marrow cell population's production of NA-CFU-c(s) (Diagram 1A) nor the NA-CFU-c(s) rate of adherence (Diagram 1C).

In light of our demonstration that the adherent cell population in this system is exclusively monocytic (see "Results," Section 1), the above data indicate that 1,25-(OH)₂D₃ inhibits mononuclear phagocyte colony formation by specifically acting on a mononuclear phagocyte precursor. With this information in hand, we turned to studying the effect 1,25-(OH)₂D₃ on authentic BMDM differentiation and began by attempting to identify an in vitro marker of mononuclear phagocyte maturation in the steroid-responsive, adherent BMDM population.

6) Characterization of 125I-Man-BSA Binding by Adherent Bone Marrow-derived Macrophages with Time in Culture—The mannose-fucose cell membrane receptor is expressed in the bone marrow exclusively by mononuclear phagocytes, and it has been demonstrated that while the more mature tissue macrophage displays an abundance of these receptors, the less mature peripheral blood monocytes do not express this cell membrane protein (24-26). Since peripheral blood monocytes are relatively immature compared to the differentiated mannose-fucose receptor-bearing tissue macrophage, we speculated that this mononuclear phagocyte-specific cell membrane receptor may serve as an in vitro marker of monocytic differentiation.

To explore the possibility that the mannose-fucose receptor appears with in vitro monocytic differentiation, we examined the binding of radiolabeled mannosylated BSA (125I-Man-BSA) to adherent BMDM with time in culture. Preliminary time course studies indicated that a minimum incubation period of 48 h is required to achieve equilibrium binding. Nonspecific binding was determined in the presence of excess yeast mannose (2 mg/ml), a competitive inhibitor of Man-fucose receptor expression by vitamin D exposure enhances expression of the mannose-fucose receptor is believed to operate primarily as an avenue for receptor-mediated endocytosis, we chose to examine the mannose-fucose receptor as a sensitive, quantitative marker of in vitro adherent BMDM differentiation and allowed us to determine specifically if 1,25-(OH)₂D₃ promotes BMDM differentiation in vitro.

7) 125I-Man-BSA Binding by 1,25-(OH)₂D₃-treated BMDM—To determine if vitamin D₃ enhances the expression of mannose-fucose cell membrane receptors, we examined binding of 125I-Man-BSA by adherent BMDM cultured in the presence or absence of 1,25-(OH)₂D₃ (50 nM) after 2, 3, and 4 days in culture. For both control and treated cells, maximal specific binding increases with time (Fig. 6A). On each day examined, however, 1,25-(OH)₂D₃-treated cells bind more ligand than do controls; these values are 0.4 versus 0.5, 0.7 versus 1.1, and 0.9 versus 1.8 ng/10⁶ cells on days 2, 3, and 4, respectively. Scatchard analysis of days 2, 3, and 4 in Fig. 6, B-D, demonstrate that the dissociation constant is unaffected by time in culture as well as exposure to the steroid. As expected from the saturation data, the estimated number of available binding sites is greater in 1,25-(OH)₂D₃-treated cells as compared to control. Furthermore, the percent increase over control binding expressed by the vitamin D-exposed cells increases progressively with time in culture, as after 4 days treated cells display 1.6 x 10⁶ receptors/cell representing a 100% increase above control. These data demonstrate that 1,25-(OH)₂D₃ enhances the number of 125I-Man-BSA binding sites on BMDM and hence indicate that this vitamin D metabolite promotes monocytic differentiation of these cells.

8) Characterization of 125I-α-Thrombin Binding to Control and 1,25-(OH)₂D₃-treated BMDM—With the knowledge that vitamin D exposure enhances expression of the mannose-fucose receptor, we next sought to determine if this regulation is "specific" to this lineage-unique protein or represents a general effect on all cell membrane receptors. As the mannose-fucose receptor is believed to operate primarily as an avenue for receptor-mediated endocytosis, we chose to examine a membrane receptor not involved in this process. α-Thrombin has been shown to exert diverse receptor-mediated effects on a variety of cell types, including the murine macrophage cell line, J774 (27, 28). In fact, this protein can induce both mitogenesis and chemotaxis in these cells (27, 29). Moreover, receptor-ligand binding studies performed with 125I-thrombin demonstrate that while the human promyleneocytic cell line HL-60 does not constitutively express α-thrombin binding sites, vitamin D₃-treated HL-60s display an abundance (30). To determine, therefore, if the 2-fold increase in mannose-fucose receptor expression manifested by vitamin D₃-treated cells is specific or representative of changes in all cell membrane receptors, we measured the effect of the steroid on binding of 125I-α-thrombin by BMDM.
1,25-Dihydroxyvitamin D₃ Modulates Macrophage Differentiation

15927

Binding studies were performed with a constant amount of ¹²⁵I-α-thrombin (0.5 µg/ml, 10⁴ cpm/mg) and increasing unlabeled α-thrombin (2-150 µg/ml). Estimations of nonspecific binding were determined by computer-assisted analyses (see “Experimental Procedures”). All binding data were then analyzed by the method of Scatchard (19).

As seen in Fig. 7A, after 4 days in culture, patterns of competition binding between radiolabeled and unlabeled α-thrombin were similar in control and vitamin D-treated cells. Conversion of these data to Scatchard analysis (Fig. 7B) reveals that exposure to vitamin D alters neither the affinity nor capacity of α-thrombin binding sites. These data demonstrate that vitamin D’s up-regulation of mannose receptor expression does not represent a global influence of this steroid on all BMDM cell membrane receptors.

DISCUSSION

This study extends our findings to authentic bone marrow-derived macrophage precursors that 1,25-(OH)₂D₃ promotes monocytic differentiation of a leukemic cell line (5). We also confirm that vitamin D inhibits mononuclear phagocyte colony formation in a dose-dependent, metabolite-specific fashion (Fig. 1), and we demonstrate that 1,25-(OH)₂D₃ inhibits colony formation independent of attendant levels of the monocyte-specific mitogen, CSF-1. Most importantly, through analysis of the steps involving colony development, we establish that the Adh-CFU-c is the target which exerts 1,25-(OH)₂D₃’s colony inhibiting influence.

To identify the target cell which vitamin D affects, we explored three phases in colony formation: 1) marrow cell production of NA-CFU-c(s), 2) adherence of the NA-CFU-c(s), and 3) proliferation and colony formation of the CFU-c (Diagram 1). Taken in total, these experiments suggest that 1) marrow cells generate NA-CFU-c(s) for at least 4 days in culture, 2) NA-CFU-c(s) adhere very rapidly (within 24 h) in culture, and 3) Adh-CFU-c(s) require approximately 6 days in culture to reach a unit size of 50 cells and thus be designated as colonies. With this knowledge we then differentially exposed cells to steroid-containing or control medium and demonstrated that vitamin D alters neither the rate of macrophage
To estimate nonspecific binding (see "Experimental Procedures"). These data were analyzed by computer-assisted mathematical analysis. Derived macrophages were incubated at 4°C with α-thrombin and increasing doses of unlabeled α-thrombin; A, competition binding for day 4 cells cultured with or without 1,25-(OH)2D3. These data were analyzed by computer-assisted mathematical analysis to estimate nonspecific binding (see "Experimental Procedures"). B, binding patterns of control versus 1,25-(OH)2D3-treated cells by the method of Scatchard Vit. D3, vitamin D3.

For example, as mentioned above, untreated HL-60 cells, which are also uncommitted to the monocytic phenotype, may also influence these less mature monocyte precursors. The influence of vitamin D on both the α-thrombin and mannose-fucose receptors is shown in Figs. 6 and 7 and, in summary, indicates that there is specific up-regulation of the monocytic differentiation-dependent receptor by vitamin D. Mannose-fucose binding in steroid-treated cells is increased in a stepwise fashion over control cells with a doubling on day 4 (Fig. 6), while in contrast, α-thrombin binding remains unchanged. Such data support the hypothesis that 1,25-(OH)2D3 promotes authentic monocytic differentiation and suggest that vitamin D-enhanced phagocytosis may be at least partially explained by an increased cell membrane expression of receptors involved in receptor-mediated endocytosis.

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REFERENCES

1,25-Dihydroxyvitamin D₃ Modulates Macrophage Differentiation