1,25-Dihydroxyvitamin D_3 Induces Collagen Binding to the Human Monocyte Line U937

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Abstract

Interactions of cells with components of the extracellular matrix can modulate cellular functions. We measured binding of a major matrix protein to U937 cells, a human promonocytic line. Radioiodinated type I or type III human collagen was bound only to U937 cells differentiated to a more mature phenotype with 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$). Binding was observed at 4°C and was saturable; Scatchard analysis of the binding to 1,25-$(OH)_2D_3$-pretreated U937 cells indicated a single class of high-affinity binding sites. Preincubation of U937 cells with interferon gamma did not induce collagen binding. Collagen binding did not appear to be dependent on fibronectin binding. Surface proteins of U937 cells were ^{125}I labeled and cell membrane proteins resolved by affinity chromatography on collagen-Sepharose. Major specifically labeled bands of 180, 155, and 125 kD were identified in membrane fractions from 1,25-$(OH)_2D_3$-pretreated U937 cells only. 1,25-$(OH)_2D_3$ appears to specifically regulate collagen binding to monocyte precursors.

Introduction

Most cells in vivo are in contact with and adhere to their extracellular matrix. Adhesiveness of cells is a fundamental property and plays a role in developmental processes such as migration and rearrangement during morphogenesis. In adult organisms in situations such as inflammation, wound healing and tumor cell invasion interactions of cells with the extracellular matrix modulate their attachment, spreading, and motility (1, 2). Collagen is a ubiquitous component of the extracellular matrix and is an important contributor to the framework of most tissues and organs. One type of cell that plays a critical role in inflammatory processes such as rheumatoid arthritis is the monocyte/macrophage. Monocytes are the major source of cytokines such as interleukin 1. Interactions with different collagens can augment production of interleukin 1 as well as prostaglandins by monocytes (3, 4).

We previously showed that human peripheral blood monocytes adhere to extracellular matrix proteins such as fibronectin and type I collagen coated on tissue culture plastic. These cells have receptors for the hormone 1,25-dihydroxyvitamin D_3 (1,25-$[OH]_2D_3$)[1] (5, 6), and incubation with 1,25-$(OH)_2D_3$ maintains their adherence to these matrices (7). Immature cells of the monocyte lineage such as the lines U937 and HL60 which also have 1,25-$(OH)_2D_3$ receptors can be induced to differentiate to a more mature phenotype as shown by adherence and spreading when they are incubated with 1,25-$(OH)_2D_3$ (8, 9). Interactions of several types of cells with the extracellular matrix are mediated at least in part by specific cellular receptors for component macromolecules (10, 11). We therefore investigated the possibility that induction of maturation in the U937 cells by 1,25-$(OH)_2D_3$ would be accompanied by the appearance of collagen receptors.

We report here that 1,25-$(OH)_2D_3$ induced specific and saturable binding of type I or type III collagen to U937 cells consistent with the presence of a membrane-associated collagen binding protein. In contrast, preincubation of U937 cells with interferon (IFN) gamma, a lymphokine that also induces certain maturational events, did not result in collagen binding. Collagen binding appeared to be independent of fibronectin binding. When solubilized membrane preparations from ^{125}I surface-labeled U937 cells were subjected to affinity chromatography on collagen-Sepharose, predominant proteins of 125, 155, and 180 kD were observed but only in the 1,25-$(OH)_2D_3$-pretreated cells. These results provide further support for the potential role of this hormone in monocyte differentiation.

Methods

Cells. U937 cells were grown in stationary suspension culture in Iscove's modification of Dulbecco's medium (IMDM, Gibco, Grand Island, NY) containing 10% fetal calf serum (FCS, Bioproducts, Inc., Warrenton, OR). Before the binding assay, cells were counted, centrifuged, and washed twice with phosphate-buffered saline (PBS) without Ca^{2+} or Mg^{2+} (Gibco). All cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2.

Reagents. 1,25-$(OH)_2D_3$ (provided by Dr. M. Uskokovic, Roche Laboratories, Nutley, NJ) was dissolved in ethanol as a stock solution of 1.6 mg/ml and diluted with medium before addition to U937 cells (final concentration, 10 ng/ml). Ethanol concentration did not exceed 0.01% and appropriate controls were performed to exclude an effect of the ethanol on collagen binding. Human recombinant IFN gamma (2×10^6 U/mg) was provided by Genentech Inc., South San Francisco, CA. IFN was purified from plasma by urea extraction (12) and fibronectin-depleted serum was a gift of Dr. R. Hynes, Massachusetts Institute of Technology, Cambridge, MA.

Preparation and iodination of collagens. Types I and III collagens were extracted from human leiomyomas as previously described (3). Type IX collagen was a gift of Dr. Daniel Herbage, CNRS, Lyon, France.

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1. *Abbreviations used in this paper:* IFN, interferon; IMDM, Iscove's modification of Dulbecco's medium; 1,25-$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3.

Collagens were labeled according to Goldberg (13) with $Na^{125}I$ (Amersham-Searle Corp., Arlington Heights, IL) by the chloramine T method to a specific activity of 10^6–10^7 cpm/μg of collagen. As shown in Fig. 1 *A*, using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) only high-molecular weight bands of collagens were labeled. There was < 5% type I collagen in the type III preparation and < 5% type III collagen in the type I preparation. The iodinated collagen preparations were not denatured based on resistance to pepsin digestion at 4°C, as shown in Fig. 1 *B*.

Binding assay. The binding assay was adapted from that of Goldberg (13) and performed with collagen in solution and U937 cells in suspension. U937 cells, usually 5×10^6, were resuspended in 1.5 ml of cold binding buffer (DMEM containing 0.1 M Hepes, pH 7.2, and 5 mg/ml of bovine serum albumin fraction [Miles Scientific Inc., Naperville, IL]) and then incubated for the indicated times in a shaking bath at 20°C with ^{125}I-collagen at the indicated concentrations. Nonspecific binding to cells was measured in replicate cultures to which 100 μg of unlabeled collagen was added. At intervals, the cells were chilled to 4°C, centrifuged, washed twice with cold binding buffer, and radioactivity of the pellet was determined. In each assay, medium without cells was processed as described to correct for binding of ^{125}I-collagen to plastic.

Cell surface labeling and membrane preparations. U937 cells were preincubated with or without 1,25-$(OH)_2D_3$ (10 ng/ml) for 60 h, then washed twice with PBS and counted. For both 1,25-$(OH)_2D_3$ pretreated and control cells, 2.2×10^8 cells were resuspended in PBS containing Ca^{2+} and Mg^{2+} at 2.2×10^7 cells/ml. The cell suspensions, 10 ml, were radioiodinated at room temperature according to Schneider et al. (14), using 2 mCi ^{125}I-sodium iodide, and lactoperoxidase (Calbiochem-Behring Corp., La Jolla, CA) at 1,175 IU/ml. The reaction was stopped after 10 min by addition of potassium iodide (400 μg/ml) and dilution with PBS. The cells were then washed three times with cold PBS with Ca^{2+} and Mg^{2+}. All subsequent steps were performed at 4°C. Cells were preswollen for 30 min in 8.5% sucrose-TEA buffer (1 mM triethanolamine hydrochloride, pH 7.4, 0.1 mM phenylmethane sulfonylfluoride), then homogenized in an ice bath with 10 strokes of a glass-teflon homogenizer. The membranes were then prepared and purified according to Mollenhauer and von der Mark (15) using a stepwise gradient of 8.5, 17, and 40% sucrose. Plasma membranes were enriched at the 17/40% interface.

Affinity chromatography and gel electrophoresis. Native type I collagen purified from human leiomyomas (the same preparation used for the binding assays) was coupled to cyanogen bromide–activated Sepharose 4B (Sigma Chemical Co., St. Louis, MO). U937 cell membranes solubilized in 0.1% Nonidet P40 (Sigma Chemical Co.) in 1 mM TEA buffer (total volume, 1.6 ml) were applied to a column containing 20 ml of native type I collagen-Sepharose at room temperature. The column was washed with 50 ml 0.1% Nonidet P40 in TEA buffer, then eluted with a linear salt gradient from 0 to 0.8 M NaCl. A total of 26 fractions of 1.5 ml each were collected. Membrane proteins in aliquots of these fractions were resolved using SDS-PAGE in slab gels with 10% acrylamide.

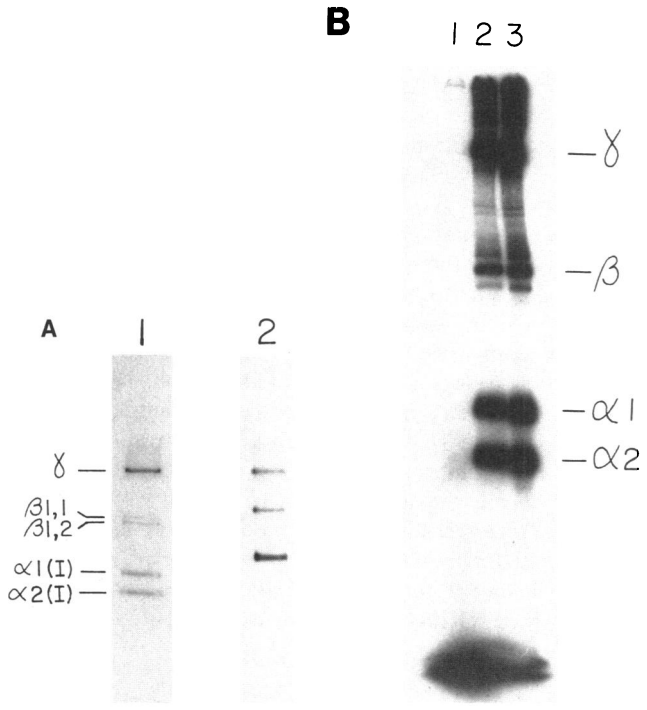

Figure 1. (*A*) SDS-PAGE of radioiodinated type I and type III collagens. Lane *1*, ^{125}I-labeled type I collagen. Electrophoresis was performed under nonreducing conditions with 5% polyacrylamide. Lane *2*, ^{125}I-labeled type III collagen. Electrophoresis was performed under reducing conditions (delayed reduction) with 5% polyacrylamide. (*B*) SDS-PAGE of ^{125}I-labeled type I collagens. Lane *1*, ^{125}I-labeled type I collagen was denatured at 45°C and treated with pepsin at 2–4°C. All the radioactivity in the form of low molecular weight species is seen at the front of the gel. Lane *2*, ^{125}I-labeled type I collagen treated with pepsin at 2°C. Lane *3*, ^{125}I-labeled type I collagen, without pepsin treatment. Resistance of ^{125}I-labeled collagen to pepsin (lane *2*) demonstrates that it has not been denatured by the iodination procedure. Electrophoresis was performed under reducing conditions with 5% acrylamide.

Results

Effects of preincubation with 1,25-$(OH)_2D_3$ and IFN gamma on specific binding of collagen type I or type III to U937 cells. Specific binding of ^{125}I-labeled type I or type III collagens to U937 cells harvested after incubation in IMDM with 10% FCS alone was not detected (Fig. 2, *A* and *B*). To investigate if binding to collagen would appear during monocyte differentiation, we preincubated U937 cells for 72 h in IMDM with 10% FCS with or without 1,25-$(OH)_2D_3$ (10 ng/ml) or for 36 h with or without IFN gamma (100 U/ml). The cells were then washed before the binding assay. In contrast to incubation in medium alone, preincubation of U937 cells with 1,25-$(OH)_2D_3$ induced specific binding of type I collagen (Fig. 2 *A*) or type III collagen (Fig. 2 *B*). No significant collagen binding was detected in U937 cells preincubated with IFN gamma (Fig. 2 *A*). Although the total bound radioactivity varied in different experiments (possibly related to differences in the specific activity of the preparation of labeled collagen and heterogeneity of the differentiation state of the U937 cells), the proportion of specific binding to total binding was reproducible. In 1,25-$(OH)_2D_3$-pretreated U937 cells, specific binding ranged from 85 to 90% of total binding. After 120 min incubation with the labeled collagen, specific binding of type I collagen was ~ 1.0% and specific binding of type III collagen was ~ 0.7% of the total added radioactivity. To further assess the specificity and reversibility of collagen binding, displacement experiments were performed. In two separate experiments, 1,25-$(OH)_2D_3$-pretreated U937 cells were incubated at 24 and 20°C, respectively, with ^{125}I type I collagen at an initial concentration of 23 pM. After 60 min of incubation with the labeled collagen alone, 1,000-fold molar excess of unlabeled type I collagen was added. In the first experiment, 100% of specifically bound collagen was displaced after 10 and 30 min of chase and in the second experiment 83% was displaced after 2 min and 100% after 10 min of chase. These results are con-

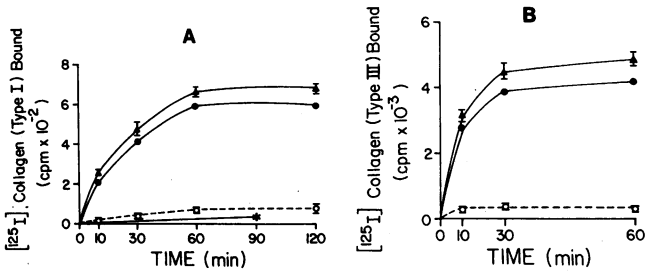

Figure 2. (*A*) Effects of preincubation with 1,25-$(OH)_2D_3$ or IFN gamma on specific binding of type I collagen to U937 cells. U937 cells were incubated for 72 h with (▲, ●) or without (○) 1,25-$(OH)_2D_3$, 10 ng/ml, for 72 h, or with IFN gamma 100 U/ml for 36 h (*) in IMDM with 10% FCS. The cells were harvested, counted, centrifuged, and 5×10^6 cells resuspended in 1.5 ml of binding buffer. ^{125}I-labeled type I collagen (100 ng, 100,000 cpm) was added, as well as unlabeled type I collagen (100 μg) to determine nonspecific binding. The cells were then incubated with the collagen in a shaking bath at 20°C for indicated times. The cells were chilled, centrifuged, washed three times in cold binding buffer, and the radioactivity of the pellet counted in a gamma counter. Points indicate means (n = 3); bars indicate SEM; (○) specific binding for U937 cells incubated with medium alone; (●) specific binding for U937 incubated with 1,25-$(OH)_2D_3$; (▲) total binding for U937 incubated with 1,25-$(OH)_2D_3$; (*) specific binding for U937 cells preincubated with IFN gamma. (*B*) Effects of preincubation with 1,25-$(OH)_2D_3$ on specific binding of collagen type III to U937 cells. U937 cells were incubated for 72 h with (▲, ●) or without (○) 1,25-$(OH)_2D_3$ (10 ng/ml) in IMDM with 10% FCS. 5×10^6 cells in 1.5 ml of binding buffer were incubated with 150 ng (150,000 cpm) of ^{125}I-labeled type III collagen, with or without 100 μg of unlabeled type III collagen, in a shaking bath at 20°C for indicated times. Bars indicate SEM; (○) specific binding for U937 cells incubated with medium alone (n = 2); (●) specific binding for U937 cells incubated with 1,25-$(OH)_2D_3$ (n = 3); (▲) total binding for U937 cells incubated with 1,25-$(OH)_2D_3$ (n = 3).

sistent with equilibrium binding of collagen to surface receptors.

To determine the length of the period of preincubation with 1,25-$(OH)_2D_3$ necessary for the induction of collagen binding, we incubated U937 cells with 1,25-$(OH)_2D_3$ for 6–72 h before the binding assay. It is shown in Fig. 3 that collagen binding was first evident between 12 and 20 h after the addition of 1,25-$(OH)_2D_3$ to the cultures and reached an apparent maximum after 36 h. As shown in Fig. 4, binding of type I

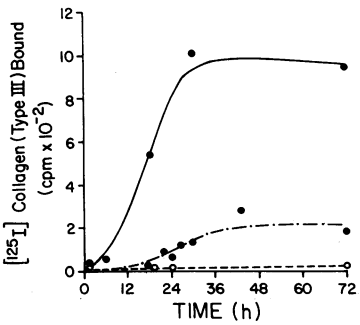

Figure 3. Induction by 1,25-$(OH)_2D_3$ of specific collagen binding to U937 cells as a function of time. U937 cells were incubated with 1,25-$(OH)_2D_3$ (10 ng/ml) in IMDM 10% FCS for indicated times. 5×10^6 cells were then incubated at 20°C for 90 min (— ● —) or 3×10^6 cells for 60 min (-·- ● -·-) with 100 ng of ^{125}I-labeled type III collagen (150,000 cpm), with or without 100 μg unlabeled type III collagen. Total and nonspecific binding were measured and specific binding calculated. Specific binding to U937 cells incubated without 1,25-$(OH)_2D_3$ (- - - ○ - - -) was also determined.

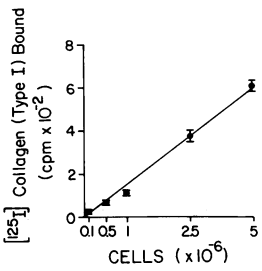

Figure 4. Binding of ^{125}I-labeled type I collagen to 1,25-$(OH)_2D_3$-pretreated U937 cells as a function of cell number. U937 cells preincubated for 72 h with 1,25-$(OH)_2D_3$ were incubated with ~ 100 ng (70,000 cpm) of ^{125}I-labeled type I collagen in 1.5 ml of binding buffer in a shaking bath at 20°C for 60 min, at the indicated cell numbers. Cells were processed as described and radioactivity of the pellet was counted. Points indicate means (n = 3), bars indicate SEM.

collagen was proportional to cell number over the range 0.1–5.0 × 10^6 cells. An increase in binding of type III collagen was also observed when more cells were used in the binding assay (Fig. 3). Assays for collagen binding were carried out at 4°C as well as 20°C using 1,25-$(OH)_2D_3$-pretreated U937 cells. Collagen binding was measurable at 4°C, although at a lower level than at 20°C (Fig. 5), suggesting that the ligand was bound to the surface of the U937 cells. Endocytosis would probably not occur at the lower temperature.

Evidence for saturability of binding and Scatchard analysis. To test whether the binding of collagen was a saturable process, we incubated 1,25-$(OH)_2D_3$-pretreated U937 cells with a fixed amount of ^{125}I-labeled type I collagen and increasing amounts of unlabeled collagen to a maximum of 100 μg. The cells were incubated at 20°C for 2 h to ensure that equilibrium conditions were achieved. Evidence that binding of type I collagen was saturable is presented in Fig. 6 *A*, where binding is plotted as a function of concentration of the ligand. Evidence for saturation after such a long (2 h) incubation time again favors the interpretation of membrane binding rather than uptake by a transport process (13). The binding data were also analyzed by the method of Scatchard (Fig. 6 *B*). The results are consistent with the presence on 1,25-$(OH)_2D_3$-pretreated U937 cells of a single class of high-affinity binding sites for type I collagen (r = 0.91); no specific binding could be calculated for U937 cells preincubated in medium alone (r = 0.28). The equilibrium dissociation constant (K_d) calculated from the slope of the line was 4.2×10^{-10} M in 1,25-$(OH)_2D_3$-pretreated U937 cells with ~ 300 binding sites per cell.

Type specificity of collagen binding. In an attempt to determine whether or not types I and III collagens were bound to different sites on the cell surface, 1,25-$(OH)_2D_3$-pretreated

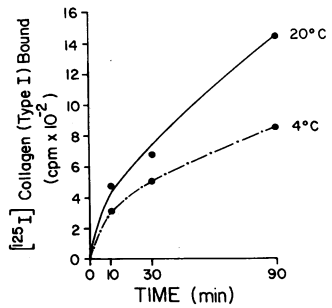

Figure 5. Binding of ^{125}I-labeled type I collagen to 1,25-$(OH)_2D_3$-pretreated U937 cells as a function of temperature. U937 cells were preincubated for 72 h with 1,25-$(OH)_2D_3$ (10 ng/ml). Aliquot portions containing 5 × 10^6 cells were then incubated with ~50 ng (150,000 cpm) of ^{125}I-labeled type I collagen, with or without unlabeled type I collagen (100 μg), in a shaking bath for 60 min, at 20°C (— ● —) or at 4°C (-·- ● -·-). Specific binding was calculated by subtracting nonspecific binding from total binding.

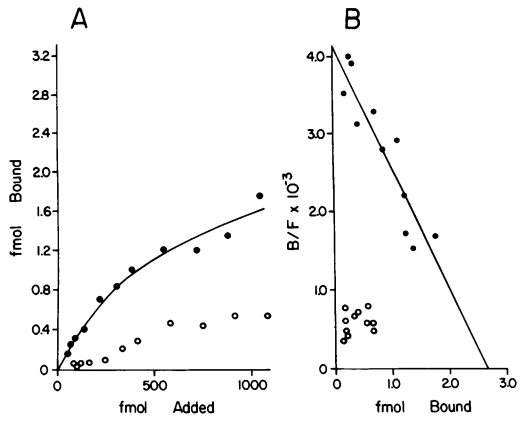

Figure 6. (*A*) Saturable binding of type I collagen by 1,25-$(OH)_2D_3$-pretreated U937 cells. Increasing amounts of unlabeled type I collagen were mixed with a fixed amount of ^{125}I-labeled collagen and added to 1,25-$(OH)_2D_3$-pretreated U937 cells (•) or control cells (○) in triplicate. The cells were then incubated at 20°C for 2 h. The bound radioactivity was measured, and femtomoles of collagen (radiolabeled and unlabeled) added and specifically bound were calculated. (*B*) Scatchard plot of ratio of bound (B) to free (F) collagen as a function of bound collagen in 1,25-$(OH)_2D_3$-pretreated U937 cells (•) and control cells (○). For 1,25-$(OH)_2D_3$-pretreated U937 cells, the Scatchard plot showed a linear fit to the data points, indicating a homogeneous class of binding sites. Equilibrium K_d calculated from the slope of the line was 4.2×10^{-10} M. The number of binding sites per cell calculated from the abscissal intercept of the line was 300 binding sites/cell in U937 cells pretreated with 1,25-$(OH)_2D_3$. No Scatchard plot binding affinity or number of binding sites could be derived in U937 cells not preincubated with 1,25-$(OH)_2D_3$.

U937 cells were incubated with a fixed amount of ^{125}I-labeled type III collagen and 100 μg of unlabeled types III or I collagens. It is shown in Table I that binding of labeled type III collagen was displaced with unlabeled type I or type III collagen. The displacement of labeled type III collagen was greater using unlabeled type III collagen compared with type I collagen. In other experiments (not shown) labeled type I collagen was displaced by unlabeled type I, type III, or type IX collagen. These results suggest that these collagens bind to similar sites on U937 cells induced with 1,25-$(OH)_2D_3$.

Effects of fibronectin and fibronectin-depleted serum on 1,25-$(OH)_2D_3$-induced collagen binding. Interactions of specific receptors on the surface of several cells with fibronectin in the extracellular matrix have been demonstrated (17–19). Fibronectin receptors are present on monocytes (20) and appear to be inducible in U937 cells (21) and HL-60 cells (22). Experiments were therefore designed to address the issue of whether induction of collagen binding by 1,25-$(OH)_2D_3$ is mediated by fibronectin binding. In other studies we could not detect fibronectin synthesized by human monocytes (7) or U937 cells (Polla, B. S., and S. M. Krane, unpublished data) preincubated with or without 1,25-$(OH)_2D_3$. In the present study U937 cells were preincubated with 1,25-$(OH)_2D_3$ (10 ng/ml, 72 h) in fibronectin-depleted serum or in normal serum. As shown in Table II, there was a mean decrease of 24% in collagen binding when U937 cells were preincubated with 1,25-$(OH)_2D_3$ in fibronectin-depleted serum. Binding assays were also performed in the presence of excess unlabeled fibronectin. No significant effects on collagen binding were observed in 1,25-$(OH)_2D_3$-pretreated U937 cells when binding was performed in the presence of 100 μg of fibronectin (Fig. 7).

Table I. Displacement of ^{125}I Type III Collagen Bound to 1,25-$(OH)_2D_3$-pretreated U937 Cells by Excess Unlabeled Type III or Type I Collagen

	^{125}I type III collagen bound	
	30-min incubation	90-min incubation
	cpm	*cpm*
^{125}I-labeled type III collagen (*150,000 cpm, 100 ng*)	4,502±122	4,736±38
+100 μg type III collagen	550±12	507±19
+100 μg type I collagen	1,223±69	1,092±39

Binding of ^{125}I type III collagen to 1,25-$(OH)_2D_3$-pretreated U937 cells was determined as described, in presence or absence of excess unlabeled type III or type I collagen. Numbers indicate means±SEM; n = 3.

Analysis of collagen-binding membrane proteins. In attempts to characterize the membrane proteins involved in collagen binding, we prepared membranes from U937 cells preincubated with or without 1,25-$(OH)_2D_3$ and surface labeled with ^{125}I. The membranes were then solubilized and applied to collagen-Sepharose affinity columns. Unbound proteins (flowthrough with TEA buffer alone) accounted for > 95% of the membrane proteins as determined by absorbance at 280 nm. Proteins bound to the collagen-Sepharose were eluted using a linear NaCl gradient and then analyzed by SDS-PAGE. Several ^{125}I-labeled proteins were detected in fractions eluted between 0.025 and 0.050 M NaCl in preparations from 1,25-$(OH)_2D_3$ pretreated U937 cells. Despite the low number of binding sites calculated by Scatchard analysis, collagen-binding proteins eluted from collagen-Sepharose columns after application of solubilized membrane preparations from 1,25-$(OH)_2D_3$ pretreated U937 cells represented ~ 3% of the applied proteins (Fig. 8 *A*). Whereas equal amounts of unlabeled unbound proteins were recovered from preparations of U937 cells which had been pretreated or not pretreated with 1,25-$(OH)_2D_3$ (Fig. 8, *A* and *B, lower panels*), the proteins eluted from the column represented < 0.5% of the applied proteins for U937 cells not pretreated with 1,25-$(OH)_2D_3$. The apparent molecular weights of the major species of proteins from 1,25-$(OH)_2D_3$-treated U937 cells which bound to collagen were ~ 125, 155, and 180 kD (Fig. 8 *A*). Lower molecular weight collagen-binding proteins were also detected (Fig. 8 *A*) in membranes from the 1,25-$(OH)_2D_3$-treated U937 cells, including a ~ 65-kD protein. These collagen-binding membrane proteins were not detected in U937 cells which had not been preincubated with 1,25-$(OH)_2D_3$ (Fig. 8 *B*).

Discussion

We found in the present study that there was no specific binding of collagen in solution to immature U937 cells, but that specific collagen binding could be induced on these cells by 1,25-$(OH)_2D_3$. This hormone primarily affects cells in bone, intestine, and kidney, where it interacts with specific intracellular receptors (23, 24). Significant numbers of receptors for 1,25-$(OH)_2D_3$ have also been measured in other normal or

Table II. Effect of Fibronectin-depleted Serum on Induction by 1,25-$(OH)_2D_3$ of Specific Collagen (Type III) Binding to U937 Cells

Preincubation condition	Mean specific binding of ^{125}I type III collagen to U937 cells			
	10-min incubation	30-min incubation	60-min incubation	120-min incubation
	cpm	*cpm*	*cpm*	*cpm*
1,25-$(OH)_2D_3$ + normal serum	1,169	1,513	1,498	1,435
1,25-$(OH)_2D_3$ + fibronectin-depleted serum	917	1,043	1,198	1,237
No 1,25-$(OH)_2D_3$ + normal serum	—	—	141	—

U937 cells were incubated with or without 1,25-$(OH)_2D_3$ for 72 h in normal or fibronectin-depleted serum. Binding assay was then performed as described in triplicate. Numbers indicate the calculated specific binding (mean nonspecific binding subtracted from mean total binding). The induction of collagen binding by 1,25-$(OH)_2D_3$ is decreased by ~ 24% (mean of the different incubation times) when the preincubation with U937 cells was performed in fibronectin-depleted serum.

neoplastic cells, particularly in mature monocytes and immature monocyte lines such as U937 and HL60 (5, 6, 25). Several functions of mononuclear cells are modulated by 1,25-$(OH)_2D_3$ including induction of maturational changes such as adherence and spreading and the appearance of surface marker proteins (7, 8, 26). Moreover, there is evidence that 1,25-$(OH)_2D_3$ enhances bone resorption by promoting the differentiation of precursor cells and increasing the number and possibly the activity of osteoclasts (9).

Collagen binding such as that observed in the present study may be a characteristic of differentiated monocytes. In preliminary experiments we have found that normal human monocytes isolated from peripheral blood also bind ^{125}I-labeled type I collagen (Polla, B. S., and S. M. Krane, unpublished data). It is possible that 1,25-$(OH)_2D_3$ plays a specific role in inducing collagen binding since IFN gamma, another factor that induces maturational changes in immature monocytes (27), does not induce this binding under the conditions of our experiments. There are other examples of developmentally or functionally regulated proteins that bind to molecules of the extracellular matrix. The tumor-promoting agent, phorbol myristate acetate, induces the synthesis and secretion of a 95-kD gelatin-binding protein in human macrophages as well as U937 cells (28, 29). Activation of platelets alters the access of the glycoprotein complex IIb/IIIa on their surface to adhesive proteins (30). Immature erythroid cells appear to lose their fibronectin receptors when they are induced to differentiate (31). During the maturation process of myocytes their capacity to interact with different matrix molecules is altered (32). The induction of collagen binding observed in U937 cells by 1,25-$(OH)_2D_3$ as reported here may reflect events that take place in cellular differentiation and migration during development and remodeling of normal tissues (33).

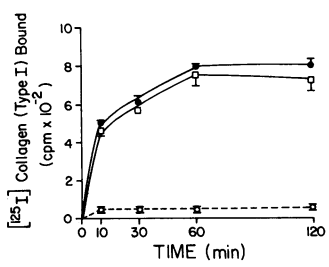

Figure 7. Comparative effects of excess unlabeled collagen or fibronectin on total collagen binding to U937 cells preincubated with 1,25-$(OH)_2D_3$. U937 cells were preincubated for 72 h with 1,25-$(OH)_2D_3$ (10 ng/ml). Aliquot portions of 5×10^6 cells were then incubated for indicated times in 1.5 ml of binding buffer, with ~ 100 ng (70,000 cpm) of ^{125}I-labeled type I collagen alone (●), with unlabeled collagen type I (100 μg) (○), or with fibronectin (100 μg) (□). Points indicate means (n = 3); bars indicate SEM.

Several characteristics of the binding reaction such as occurrence at 4°C, specificity, saturability, and kinetics are consistent with those of a membrane-associated collagen-binding protein. In earlier reports it had been suggested that fibronectin is a required mediator for attachment of monocytes to collagen (34, 35). It is unlikely, however, that the collagen binding to U937 cells induced by 1,25-$(OH)_2D_3$ that we observed is mediated by fibronectin. First, we could not detect fibronectin by SDS-PAGE in extracts of U937 cells regardless of whether they had been preincubated with 1,25-$(OH)_2D_3$. Second, when U937 cells were preincubated with 1,25-$(OH)_2D_3$ in fibronectin-depleted serum, although the induction of collagen binding was decreased by ~ 24% it was not abolished (Table II). Third, excess unlabeled fibronectin did not compete with labeled collagen binding (Fig. 7). Finally, although 1,25-$(OH)_2D_3$ induced adherence of U937 cells to fibronectin-coated surfaces, there was no significant binding of ^{125}I-labeled fibronectin in solution to U937 cells (Healy, A. M., B. S. Polla, and S. M. Krane, unpublished data). These results do not exclude a role for fibronectin in collagen binding but suggest that 1,25-$(OH)_2D_3$ induces a collagen-binding protein(s) distinct from fibronectin-binding proteins. Collagen-binding protein(s) distinct from fibronectin-binding proteins have also been described in other cells such as chondrocytes (15, 36), platelets (37), hepatocytes (38), fibroblasts (39), endodermal cells (40), and osteosarcoma cells (41). We detected the presence of proteins accessible to lactoperoxidase-catalyzed iodination at the surface of U937 cells (thus membrane-associated), which were induced by 1,25-$(OH)_2D_3$ that interacted with type I collagen covalently linked to Sepharose and was eluted at 0.025–0.050 M NaCl. These characteristics are consistent with those of specific collagen-binding proteins. The collagen-binding protein from chondrocytes described by Mollenbauer and von der Mark (15) has a different molecular weight but is eluted from collagen affinity columns at similar salt concentrations. This relatively weak type of binding has been described previously for other adhesion receptors. If monocytes were to use such cell surface–binding proteins in migration through vessel walls, they would be readily detachable from the substrate.

The fibronectin receptor, the vitronectin receptor, and the platelet membrane glycoprotein IIb/IIIa are members of the family of receptors that recognizes the Arg-Gly-Asp-(RGD) sequence in the ligand and are composed of two subunits with

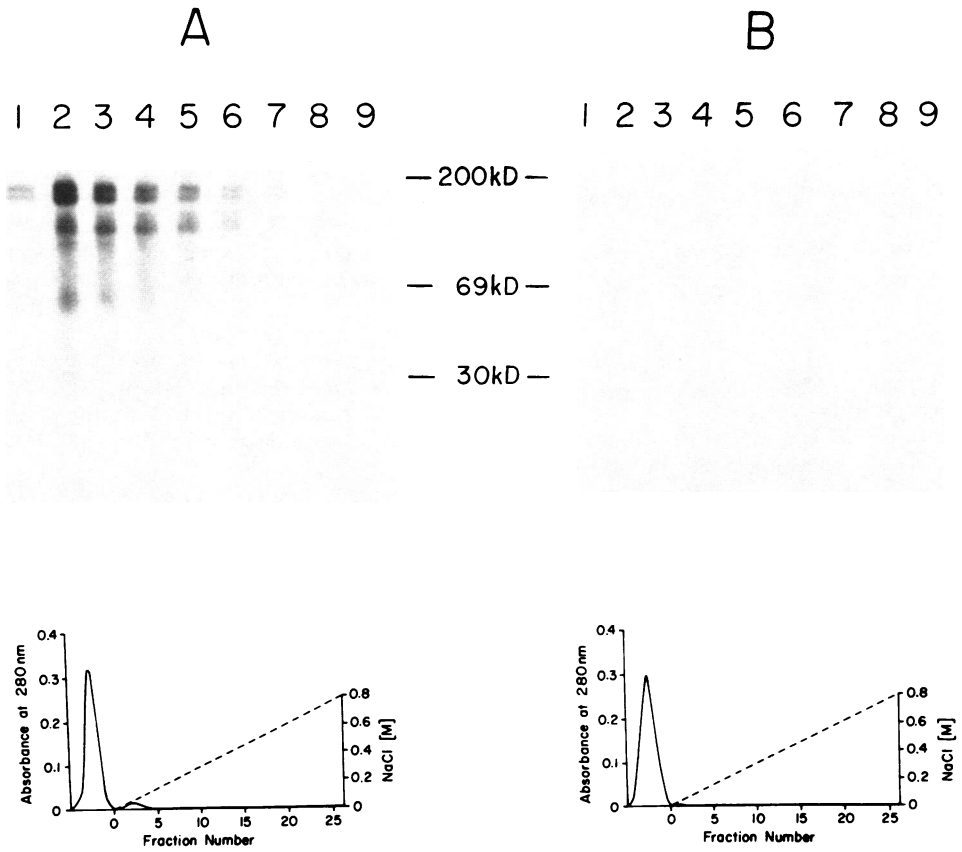

Figure 8. Purification of collagen-binding proteins by affinity chromatography of U937 cell membranes on type I collagen-Sepharose. Plasma membranes were solubilized as described and passed over affinity columns of type I collagen coupled to Sepharose 4B. Unbound proteins were removed from the column by TEA buffer wash (total volume, 50 ml). Bound proteins were eluted with a linear (0–0.8 M) NaCl gradient, and 26 fractions of 1.5 ml each were collected. Aliquot portions of fractions 1–9 were analyzed by SDS-PAGE with 10% polyacrylamide (lanes *1–9*). (*A*) U937 cells pretreated with 1,25-$(OH)_2D_3$. (*B*) Control U937 cells. Both gels were exposed to Kodak X-Omat AR film for 2 wk.

a characteristic electrophoretic pattern after reduction, consistent with a related pattern of intrachain disulfide bonding (11, 17, 42). In preliminary experiments, the 125, 155, and 180-kD collagen-binding proteins we describe did not show this characteristic response to reduction (Polla, B. S., and S. M. Krane, unpublished data). Several RGD sequences are present in the type I collagen molecules in both the $\alpha 1$ and the $\alpha 2$ chains (43), and Dedhar et al. have recently demonstrated that a cell surface receptor complex on osteosarcoma cells can recognize an RGD sequence on type I collagen (41). We cannot exclude that the collagen-binding proteins induced by 1,25-$(OH)_2D_3$ could recognize such RGD sequences although they appear to be of different molecular weight than those described. It is possible, however, that the low abundance labeled 65-kD protein could be related to the 70-kD protein reported by Chiang and Kang (37) and Dedhar et al. (41) although they were of different affinity based on the concentration of NaCl required for elution from collagen-Sepharose affinity columns (41). If the collagen-binding proteins on U937 cells induced by 1,25-$(OH)_2D_3$ do recognize RGD sequences, this recognition must be dependent upon adjacent sequences (44) or protein conformation (43), because fibronectin does not compete with collagen binding. Both fibronectin and vitronectin receptors recognize RGD sequences, but exhibit mutually exclusive reactivities toward fibronectin or vitronectin (42). In any case, the accessible portion of the collagen molecule recognized by the collagen-binding protein(s) must be at least partially shared by collagen types I, III, and IX, the types tested in our experiments.

The apparent molecular weights of two of the induced collagen-binding proteins (180 and 155 kD) are consistent with those of the α-chains of the leukocyte surface antigens LFA-1 and p150-95 (45). LFA-1 however has been reported to be present on immature U937 cells and decreases after induction of differentiation with phorbol esters (45), rendering unlikely the possibility that the collagen-binding proteins induced by 1,25-$(OH)_2D_3$ belong to this family of adhesion molecules. Moreover, no band compatible with the α-chains of these molecules (95 kD) was observed in our preparations.

Interactions between cells and extracellular matrix molecules, if receptor-mediated, would necessitate the presence of the receptor on the cell surface, whereas adherence and spreading would require in addition the redistribution of these receptors (46). 1,25-$(OH)_2D_3$ not only induces collagen binding to U937 cells but also their adherence to tissue culture plastic. The adherence of U937 cells to collagen as compared with tissue culture plastic, however, is not increased by pretreatment with 1,25-$(OH)_2D_3$ (Polla, B. S., and S. M. Krane, unpublished data) and control U937 cells do not adhere to collagen at all (21, 47). The relatively low number of collagen-binding sites ($\sim$ 300/cell) induced on U937 cells by 1,25-$(OH)_2D_3$ compared with $\sim 5 \times 10^5$ sites/cell reported for fibroblasts (13, 19), may account for the lack of adherence to collagen. 1,25-$(OH)_2D_3$ also induces several other surface proteins on U937 cells that could conceivably interfere with the redistribution of the binding sites (8, 46). Finally, binding of cells to molecules in solution and adherence to the same molecules immobilized on a surface may be separate events.

Interactions of cells with the extracellular matrix support their growth, adherence, spreading, and locomotion (1, 48), and interactions of monocyte macrophages with their extracellular matrix may be involved in inflammation and skeletal

remodeling. Several effects of collagens on monocyte functions such as synthesis of interleukin 1 and prostaglandins (3, 4) were referred to earlier. It has also been shown that monocytes cultured on collagen matrices as contrasted with glass or other coatings are more highly phagocytic and more closely resemble resident tissue macrophages (49). Characterization of the 1,25-$(OH)_2D_3$-induced cell surface collagen-binding proteins and comparison with other putative receptors for collagen could provide further understanding of cell interactions with this most abundant structural protein.

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