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Transforming growth factor β enhances integrin expression and type IV collagenase secretion in human monocytes

(recruitment/inflammation/metalloproteinases/growth factors/adhesion)

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ABSTRACT **Transforming growth factor β (TGF-β), secreted within an inflammatory site or injected locally, induces leukocyte margination, chemotaxis, and accumulation. In addition to its potent direct chemotactic activity, TGF-β may promote this leukocyte response by influencing cell surface integrin expression. At picomolar concentrations, TGF-β increases steady-state mRNA levels for both the α_5 and the β_1 chain of the fibronectin receptor in human blood monocytes. This increase in gene expression is reflected by selectively enhanced expression of α_5 (CDw49e), β_1 (CDw29), and also α_3 (CDw49c) adhesion molecules on the cell surface. Functionally, TGF-β promotes, in a dose- and time-dependent fashion, monocyte adhesion to type IV collagen, laminin, and fibronectin. Potentially facilitating the movement of monocytes through the extracellular matrix, TGF-β triggers transcriptional and posttranscriptional regulation of both the 92-kDa and the 72-kDa gelatinase/type IV collagenase. Thus, TGF-β may play a pivotal role in the early phases of inflammation and repair through its ability to mediate monocyte adhesion, chemotaxis, and enzymatic digestion of extracellular matrix, whereas in chronic lesions, excess TGF-β may contribute to persistent leukocyte accumulation.**

Evolution of inflammatory and immune reactions is dependent upon the recruitment and migration of circulating leukocytes to sites of injury or antigen deposition. The accumulation of leukocytes is dependent not only on chemotactic signals emanating from the inflammatory site but also on cell–cell and cell–matrix interactions (1). Many of these cellular and matrix interactions are dependent upon expression of adhesion molecules which facilitate targeting and retention of circulating cells to sites of immunologic challenge (2–6). Recent evidence indicates that transforming growth factor β (TGF-β), an extremely potent chemotactic factor *in vitro* (7, 8), profoundly influences local leukocyte accumulation when injected *in situ* (9–11). Whether this recruitment of leukocytes is due solely to its chemotactic potential or is the consequence of additional mechanisms is unknown. No studies have yet addressed the ability of TGF-β to influence the requisite adhesion of primary leukocytes to endothelium and to extracellular matrix in their journey from the circulation to the tissues.

Integrins represent a family of cell surface αβ heterodimeric proteins that mediate cell adhesion to other cells and to the extracellular matrix (2–6, 12, 13). Functional adhesion molecules have been shown to be vital to platelet aggregation, wound healing, immune activation, and tissue repair and also in tumor invasion (reviewed in ref. 4). The widespread role of these adhesion molecules, not only in fundamental biological processes, but also in inflammation, has prompted the design of therapeutic modalities aimed at regulating cell adhesion (4, 5).

In this study, we explore whether TGF-β, which is released by platelets early in an inflammatory response (14), and subsequently by inflammatory cells themselves (15–17), can influence integrin expression on mononuclear phagocytic cells. The ability of TGF-β to rapidly promote increased α and β integrin subunit expression on monocytes, accompanied by augmented cell binding to extracellular matrix constituents, including the basement membrane proteins laminin and type IV collagen, as well as fibronectin, implicates this cytokine in coordinating the early margination, recruitment, and migration of leukocytes to an inflammatory site. Moreover, TGF-β stimulation of monocytes to secrete the 92-kDa and 72-kDa forms of type IV collagenase/gelatinase, crucial to proteolytic degradation of basement membrane proteins (18, 19), further supports the role of this cytokine in facilitating monocyte movement from the circulation through the tissues.

MATERIALS AND METHODS

Peripheral Blood Mononuclear Cells. Mononuclear leukocytes were obtained by density gradient centrifugation of heparinized blood collected by leukapheresis (Department of Transfusion Medicine, National Institutes of Health). Purified populations of nonadherent, nonactivated monocytes (>90% $CD14^+$, $CD25^-$) were obtained by counterflow centrifugal elutriation (20), suspended at 5×10^6 per ml in Dulbecco's modified Eagle's medium (DMEM) (Mediatech, Washington, DC) with gentamicin (50 μg/ml) and glutamine (2 mM), and cultured in polypropylene tubes (17 × 100 mm; Falcon, Becton Dickinson) with human or porcine TGF-β_1 (R & D Systems, Minneapolis) at 0.01–30 ng/ml or lipopolysaccharide (LPS, *Escherichia coli* 055:B5, Sigma) at 1 μg/ml. The highest concentration of TGF-β used in the cultures contained <6 pg of endotoxin per ml (limit of detection) as determined by the *Limulus* amoebocyte lysate assay (courtesy of Don Hochstein, Food and Drug Administration, Bethesda, MD).

Fluorescence Binding Studies. Monocytes (1×10^7 per ml in phosphate-buffered saline with 0.1% sodium azide) were incubated at 4°C for 30 min with monoclonal antibodies to the relevant integrin subunits: β_2 (CD18), β_3 (CD61), α_2 (CDW49b), and α_3 (CDw49c) (Telios Pharmaceuticals, San Diego and AMAC, Westbrook, ME); α_5 (CDw49e, mAb16) and β_1 (CDw29, mAb13) generously provided by K. Yamada (National Institute of Dental Research) (21). The cells were washed, incubated with fluorescein-labeled goat anti-mouse IgG (Tago) or goat anti-rat IgG (HyClone), washed twice, and resuspended in 2% paraformaldehyde until analysis with the FACScan (Becton Dickinson) (20).

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Abbreviations: TGF, transforming growth factor; LPS, lipopolysaccharide.

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Cell Attachment Assays. Tissue culture wells (24-well plates, Costar) were coated with 1 μg of bovine serum albumin (Boehringer Mannheim) or laminin, collagen IV, or fibronectin (22–25) in 310 μl of water, which was evaporated overnight. Gelatin (1% in 0.5 ml of water) (Bio-Rad) was added and the plates were incubated at 37°C. After 1 hr, the solution was aspirated. Monocytes in DMEM without serum were seeded in coated wells at 2.5×10^5 cells per 0.5 ml. After incubation for 15 min at 4°C, the unattached cells were removed by washing the wells twice with phosphate-buffered saline, and the attached cells were stained with Diff Quik (Baxter Scientific Products, McGraw Park, IL). Monocytes were quantitated in triplicate 1.5-mm^2 fields per well (×10 magnification). Statistical significance was determined by the Scheffe *F* test.

Substrate Specificity for Monocyte Attachment. TGF-β-treated cells (5×10^4) were cultured on fibronectin substrates in the presence or absence of competing peptide (100 μg/0.5 ml): Gly-Arg-Gly-Asp-Ser (GRGDS), containing the amino acid sequence RGD; GRGES, containing the RGE sequence, or DELPQLVTLPHPNLHGPEILDVPST, the CS-1 connecting segment peptide from an alternatively spliced region of fibronectin (kindly provided by K. Yamada) (26). In additional experiments, TGF-β-treated monocytes were plated on fibronectin in the presence of antibodies (100 μg/0.5 ml): control IgG (rat) or monoclonal antibodies to the β_1 (mAb13) or α_5 (mAb16) integrin subunits (21). After incubation, the numbers of attached cells were determined as above.

Collagenase Assay. Release of gelatinase/type IV collagenase into the monocyte supernatants (5×10^6 monocytes per ml, 18-hr cultures) was monitored by zymographic analysis. Serum-free supernatants were dialyzed against 1 mM Tris, pH 8.0/0.5 mM $CaCl_2$, lyophilized, and suspended in distilled water before addition to electrophoretic sample buffer without reducing agents or heating. Zymography was performed in 10% polyacrylamide slab gels containing 1% gelatin (18). After electrophoresis, the slabs were washed in 2.5% Triton X-100 for 1 hr, equilibrated in 10 mM Tris (pH 8.0) for 30 min, and incubated at 37°C in 50 mM Tris, pH 8.0/0.5 mM $CaCl_2$/1 μM $ZnCl_2$. The gels were stained with 0.2% Coomassie blue and destained with 7% acetic acid. Cleavage rate estimates were obtained by scanning the gels with a computing densitometer (Shimadzu, Columbia, MD).

RNA Analysis. Total RNA was prepared from 15×10^6 monocytes by acid guanidinium thiocyanate–phenol–chloroform extraction (27). For Northern blots, 5 μg of total RNA was fractionated in 1.0% agarose/formaldehyde gels, transferred to nitrocellulose filters, and prehybridized. The blots were hybridized overnight with ^{32}P-labeled cDNA for fibronectin receptor α_5 and β_1 subunits (28) (Telios), 72-kDa and 92-kDa gelatinase/type IV collagenases (18, 19) (generously provided by G. Goldberg, Washington University, St. Louis), and pHe7 (J. Reed, University of Pennsylvania) (29). The filters were exposed to phosphor screens for 4–18 hr and analyzed for mRNA bands with the PhosphorImager system (Molecular Dynamics, Sunnyvale, CA) using Image Quant software and a Hewlett–Packard III laser printer. Scanned images were produced utilizing Adobe Photoshop (Adobe Systems, Mountain View, CA), a Microtek MSF-300ZS flatbed image scanner (Microtek International, Hsinchu, Taiwan), and a Phaser CP color printer (Tektronix).

RESULTS

TGF-β Augmentation of Binding of Blood Monocytes to Matrix Proteins. Monocytes were cultured in suspension with TGF-β (0.01–30 ng/ml) for 18 hr, washed, and incubated on matrix proteins. No increased adhesion was evident until concentrations ≥0.3 ng/ml were added to the monocytes (Fig. 1*A*). However, at 1–10 ng/ml, ≥20 times as many of the TGF-β-treated monocytes bound to fibronectin as the controls (9 ± 3 cells per field). At concentrations exceeding 10 ng/ml, decreased binding was evident. Although increased binding levels were variable between individual donors because of differing levels of constitutive binding, TGF-β always increased monocyte–matrix interactions above that of untreated control cells. Similar enhancement of binding to type IV collagen (Fig. 1*B*) and to laminin (Fig. 1*C*) occurred when the monocytes were treated with increasing concentrations of TGF-β.

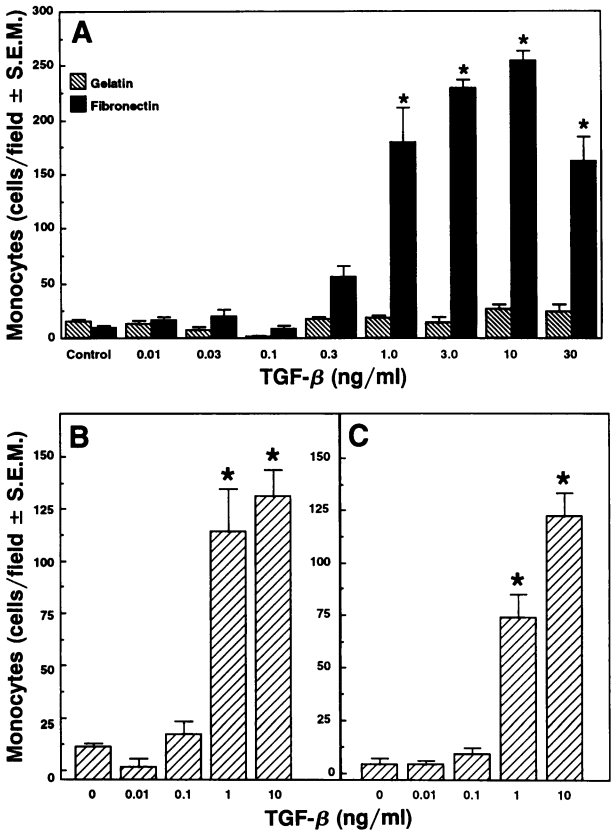

FIG. 1. Attachment of TGF-β-treated monocytes to matrix substrates. Control or TGF-β treated monocytes (2×10^5) were added to replicate wells coated with gelatin or fibronectin (*A*), type IV collagen (*B*), or laminin (*C*). After 15 min at 4°C, unattached cells were removed and attached cells were counted in three 1.5-mm^2 fields per well. Data represent the mean number of cells per field ± SEM. Significance was determined at 95% by the Scheffe *F* test and significant differences from untreated cells are indicated by asterisks.

Increased binding to fibronectin was observed between 4 and 8 hr after monocyte exposure to TGF-β, reaching maximal levels by 12–16 hr (Fig. 2). A parallel kinetic response was demonstrated for TGF-β-induced monocyte attachment to type IV collagen and laminin substrates (data not shown).

Specificity of Monocyte Binding to Fibronectin. TGF-β-treated and control monocytes were washed and added to a fibronectin substrate in the absence or presence of domain-specific competitor peptides. Although the RGE and CS-1 peptides did not block TGF-β-treated monocyte attachment to fibronectin, the RGD peptide inhibited binding to control levels (Fig. 3*A*), documenting the role of the RGD binding domain in monocyte attachment.

Inclusion of antibodies to the α_5 subunit with the TGF-β-treated monocytes during incubation in the attachment assay blocked monocyte binding >50%, and β_1-subunit antibodies completely inhibited (284 ± 9 vs. 57 ± 7 cells) monocyte–fibronectin interactions, to even below control levels (Fig. 3*B*). Incubation with control IgG had no inhibitory effect. These data implicate the increase in α_5 and β_1 molecules on monocytes exposed to TGF-β as being important in the observed increased adhesion to extracellular matrix proteins.

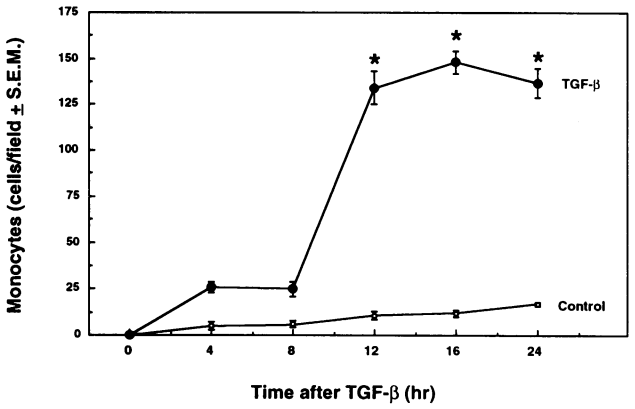

FIG. 2. Kinetics of monocyte attachment to fibronectin after stimulation with TGF-β. Monocyte attachment was determined as described in Fig. 1 following 4–24 hr of culture without (control) or with TGF-β at 1 ng/ml. Significant differences from the untreated group are indicated by asterisks.

Integrin Expression by TGF-β-Treated Monocytes. Flow microfluorometric analysis showed that monocytes constitutively expressed both β_1 (CDw29) and β_2 (CD18) integrin molecules, which increased after exposure to picomolar levels of TGF-β (β_1, Fig. 4). In contrast, the specificity of this TGF-β-mediated response was apparent by the absence of modulation of the β_3 (CD61) subunit. Subsequent flow microfluorometric analysis revealed that TGF-β also selectively influenced α subunit expression, in that TGF-β had no effect on α_2 (CDw49b) (3% for control vs 4% for TGF-β), but both α_3 (CDw49c) and α_5 (CDw49e) subunits were increased (Fig. 4). Thus, consistent with their adhesion responses, TGF-β-treated monocytes express increased $\alpha_5\beta_1$, the functional receptor for the RGD sequence of fibronectin, and increased $\alpha_3\beta_1$, which also interacts with laminin and type IV collagen.

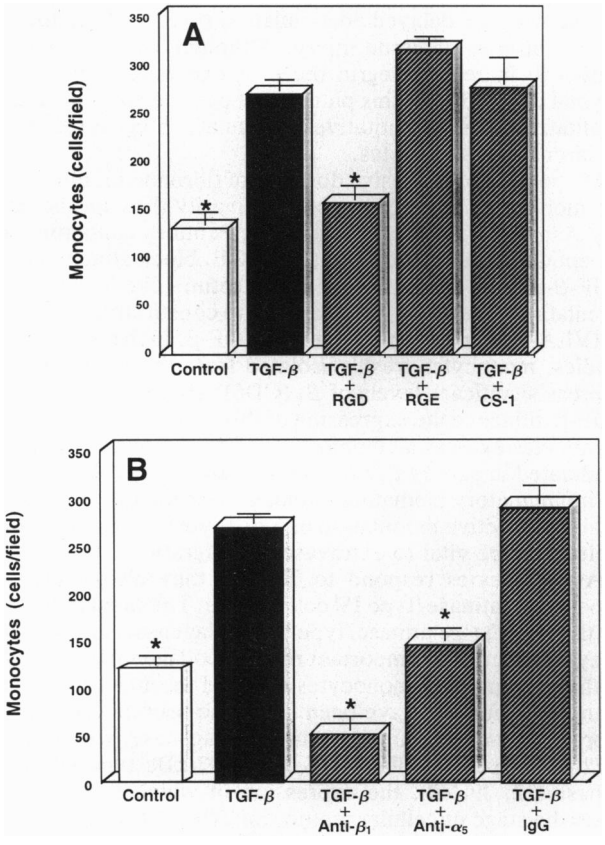

FIG. 3. Specificity of TGF-β-stimulated monocyte attachment to fibronectin. (*A*) Inhibition of attachment to fibronectin by competition with synthetic peptides. Attachment assays were carried out in the presence or absence of 100 μg of GRGDS (RGD), GRGES (RGE), or CS-1 peptide. Significant differences from TGF-β-treated cells are indicated by asterisks. (*B*) Inhibition of attachment to fibronectin by antibodies to the β_1 and α_5 integrin subunits. Attachment of TGF-β-treated monocytes to fibronectin was determined in the presence or absence of 100 μg of the indicated antibody or control IgG. Significant differences from TGF-β-treated cells without immunoglobulin are indicated by asterisks.

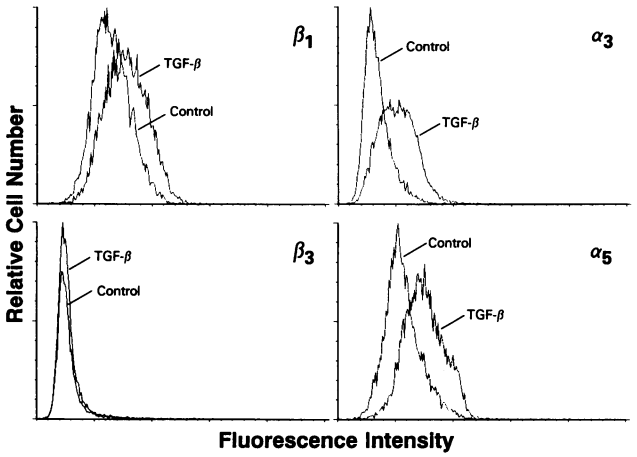

FIG. 4. Effect of TGF-β on monocyte integrin expression. Monocytes cultured in the absence or presence of TGF-β (10 ng/ml) for 22 hr were incubated with monoclonal antibodies to β_1 (mAb13), β_3, $\alpha 3$, or α_5 (mAb16), washed, and incubated with fluorescein-conjugated goat anti-mouse IgG or goat anti-rat IgG before analysis by flow microfluorometry. Data are from a representative experiment (n = 6).

Effect of TGF-β on Monocyte Fibronectin Receptor mRNA. To differentiate between potential receptor activation and/or increased protein expression, total RNA from control and TGF-β-treated monocytes was probed by Northern analysis for α_5 and β_1 mRNAs (Fig. 5). Interestingly, TGF-β appeared to differentially regulate α_5 and β_1 subunit mRNA levels in that within 90 min a definitive dose-dependent increase in steady-state mRNA levels for the α_5 chain was apparent. Although TGF-β at 0.1 ng/ml induced substantial mRNA expression, 1–10 ng/ml was usually optimal for α_5 chain induction. A transient increase in α_5 mRNA was occasionally also seen in the control populations 2–4 hr after the onset of the incubation period, but as shown at 12 hr the TGF-β-treated cells always had higher mRNA levels (Fig. 5). In contrast to α_5 mRNA expression, however, the β_1 response was delayed, with no significant increase at 90 min, a

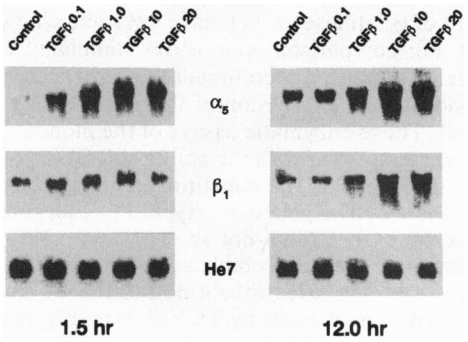

FIG. 5. TGF-β regulation of integrin gene expression. Monocytes were cultured with TGF-β (0.1–20 ng/ml) for 1.5 or 12 hr before RNA was isolated and probed by Northern analysis for α_5, β_1, and He7 mRNA expression. This experiment is representative of four.

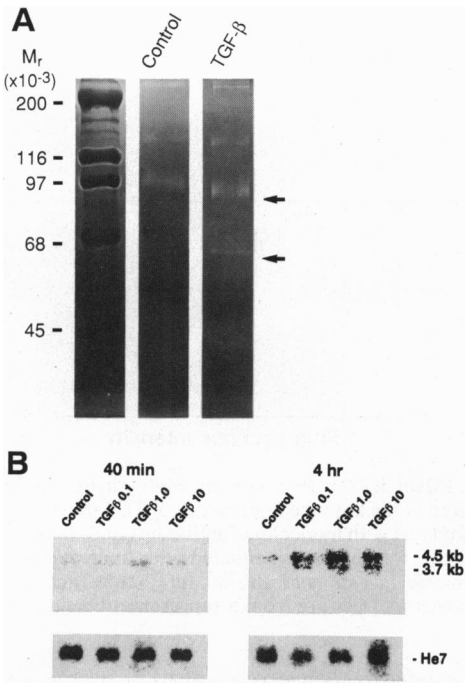

FIG. 6. TGF-β induction of gelatinase/type IV collagenase production. (*A*) Zymogram analysis of TGF-β-treated monocyte supernatants. Monocytes (5×10^6 per ml) were cultured with TGF-β (1–20 ng/ml) for 18 hr. The culture supernatants were dialyzed and lyophilized before electrophoresis in a gelatin-containing 1% polyacrylamide gel. Size markers are indicated (left lane). (*B*) RNA obtained at 40 min and 4 hr after TGF-β stimulation was probed for mRNAs encoding the 72-kDa enzyme and He7.

marginal increase at 4–8 hr, and maximal levels at 8–12 hr after exposure to TGF-β (Fig. 5).

TGF-β-Mediated Collagenase Production. An important component of leukocyte movement from the circulation into the tissues requires passage through extracellular matrices. To determine whether TGF-β also influences this aspect of monocyte migration, supernatants were analyzed for the presence of protease activity. Whereas control monocyte supernatants contained low levels of 92-kDa enzyme, no detectable 72-kDa enzyme activity was identified by zymographic analysis of the gelatin substrate (Fig. 6*A*). By comparison, parallel supernatants from monocytes incubated with TGF-β (10 ng/ml) contained a 65-kDa gelatinase/type IV collagenase, consistent with the activated form of the 72-kDa enzyme. Moreover, an increase in activity (170% by densitometric analysis) of the 92-kDa gelatinase occurred after stimulation of the cells with TGF-β. TGF-β also appeared to influence expression of larger forms ($\approx$120 kDa and >200 kDa) of gelatinase, which have yet to be characterized (Fig. 6*A*). For comparison, monocytes stimulated with LPS demonstrated increased secretion of the 92-kDa enzyme, but LPS did not induce expression of the 72-kDa enzyme (data not shown). These enzymatic assays of the monocyte supernatants were consistent with the ability of TGF-β to increase enzyme mRNA levels. The constitutive steady-state mRNA level for the 92-kDa gelatinase/type IV collagenase was augmented by TGF-β (data not shown), and mRNA for the 72-kDa enzyme, barely detectable at 30–60 min, was strongly induced by 4 hr (Fig. 6*B*). In addition to the 4.5-kb band, a 3.7-kb mRNA was induced by TGF-β, possibly as a consequence of alternative splicing.

DISCUSSION

The ability of TGF-β to induce leukocyte accumulation when injected *in situ* (9–11) suggests mechanisms, in addition to a concentration-dependent chemotactic stimulus (7, 8), whereby TGF-β may promote emigration of circulating cells into tissues. In this study, we demonstrate that in addition to chemotaxis, TGF-β influences monocyte recruitment and accumulation via at least three interrelated pathways: (*i*) increased expression of α and β integrin subunit molecules, (*ii*) augmented cell–matrix interactions, and (*iii*) collagenolytic activity.

Freshly isolated human peripheral blood monocytes exposed to TGF-β in suspension cultures demonstrate increased expression of specific α and β_1 integrin subunits, which represent the predominant class of extracellular matrix receptors. The increase in $\alpha_3\beta_1$ (VLA-3) and $\alpha_5\beta_1$ (VLA-5) integrins reflects a corresponding increase in attachment to extracellular matrix proteins. Whereas $\alpha_3\beta_1$ exhibits specificity for laminin, type IV collagen, and fibronectin, $\alpha_5\beta_1$ uniquely interacts with fibronectin (6). Monocytes constitutively express fibronectin receptors (30, 31), and the increased attachment of TGF-β-treated cells to purified fibronectin matrices may represent functional changes in the receptors as well as increased expression. Although activation of mononuclear phagocytes by γ-interferon and LPS has been shown to increase adherence to laminin and type IV collagen (32), increased fibronectin receptor expression in response to these inflammatory stimuli is less consistent (31, 32).

Interestingly, TGF-β does not appear to mediate an immediate change in adhesiveness, but the kinetics are compatible with the delayed accumulation of cells of monocytic lineage at sites of tissue injury. Although TGF-β has been shown to increase integrin receptor expression on mesenchymal cells (33–35), this pluripotent peptide also appears to qualitatively and quantitatively modulate integrin receptors on circulating leukocytes.

Of the multiple adhesive domains of fibronectin, binding of the monocytes appears to occur primarily through the Arg-Gly-Asp (RGD) cell recognition site. Peptides containing the tripeptide sequence RGD, but not RGE, block attachment of TGF-β-treated monocytes to fibronectin. Like T cells (36), circulating monocytes do not appear to constitutively express α_2 (VLA-2), nor is α_2 influenced by TGF-β. In these and other studies, monocytes freshly isolated from the blood did not express significant levels of β_3 (CD61) (6, 37, 38), nor does TGF-β influence the expression of this subunit. Thus, human monocytes express a combination of VLA integrins that are modulated in part by the activation state of the cell, and also by inflammatory mediators. Among these mediators, TGF-β exhibits selective modulation of monocyte integrin receptors which may be vital to extravascular migration.

As monocytes respond to TGF-β, they also appear to generate gelatinase/type IV collagenase. The rapid induction of the 72-kDa gelatinase/type IV collagenase mRNA and enzyme suggests an important role for collagenolytic activity in the movement of monocytes into and through the tissues. Human monocytes have been shown to secrete four metalloproteinases, including interstitial collagenase, stromelysin, a 72-kDa type IV collagenase, and a 92-kDa type IV collagenase (22, 39, 40), the expression of which is dependent upon the stage of cellular maturation (40). Although production of the 92- and 72-kDa type IV collagenases increases markedly following adherence and differentiation of monocytes into macrophages (40, 41), TGF-β appears to stimulate undifferentiated monocytes to transcribe and translate the 72-kDa enzyme while concurrently augmenting the constitutive levels of the 92-kDa enzyme. Previous studies have shown that the 72- and 92-kDa messages are 3.1 and 2.8 kb in size, respectively (18, 19). However, in our system, monocytes expressed a 4.5-kb and a 3.7-kb species of the 72-kDa mRNA after stimulation with TGF-β (Fig. 6*B*). Whether this latter species is the result of alternative splicing

is not known. A 3.1-kb mRNA was apparent when the Northern blots were probed with the 92-kDa cDNA (data not shown).

Although TGF-β clearly inhibits metalloproteinases in fibroblasts (42, 43), it has been found to increase type IV collagenase in cultured human keratinocytes and fibroblasts (42, 44). Differential secretion of these enzymes by circulating and stimulated monocytes may contribute to establishment of a pathway through basement membrane and extracellular matrix. Type IV collagen, the major structural component of basement membranes, serves as a barrier which cells must degrade to facilitate their passage from the circulation into the tissues, and TGF-β, rapidly deployed at an inflammatory site, may be responsible for initiating these events. In chronic inflammatory sites, the continuous release of TGF-β by activated inflammatory cells may provide a self-perpetuating mechanism for the recruitment of new populations of cells into the lesions (45, 46). Persistence of an inflammatory stimulus favors excessive recruitment of inflammatory cells with the potential for leukocyte-mediated tissue damage as the basis of chronic autoimmune and inflammatory diseases. By contrast, deficiencies in these pathways may be responsible for aberrant host defense and recurrent life-threatening infections. It is clear that carefully regulated leukocyte recruitment from the circulation to sites of inflammation is an essential component of host defense, and TGF-β may be a key factor in orchestrating these events.

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1. Wahl, S. M. (1992) in *Encyclopedia of Immunology* eds. Roitt, J. M. & Delves, P. J. (Saunders, London), pp. 1304–1307.
2. Springer, T. (1990) *Nature (London)* **346,** 425–434.
3. Albelda, S. M. & Buck, C. A. (1990) *FASEB J.* **4,** 2668–2680.
4. Ruoslahti, E. (1991) *J. Clin. Invest.* **87,** 1–5.
5. Osborn, L. (1990) *Cell* **62,** 3–6.
6. Hemler, M. E. (1990) *Annu. Rev. Immunol.* **8,** 365–400.
7. Wahl, S. M., Hunt, D. A., Wakefield, L., McCartney-Francis, N., Wahl, L. M., Roberts, A. B. & Sporn, M. B. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5788–5792.
8. Brandes, M. E., Mai, U. E. H., Ohura, K. & Wahl, S. M. (1991) *J. Immunol.* **147,** 1600–1606.
9. Allen, J. B., Manthey, C. L., Hand, A. R., Ohura, K., Ellingsworth, L. & Wahl, S. M. (1990) *J. Exp. Med.* **171,** 231–247.
10. Roberts, A. B., Sporn, M. B., Assoian, R. K., Smith, J. M., Roche, N. S., Wakefield, L. M., Heine, U. I., Liotta, L. A., Falanga, V., Kehrl, J. H. & Fauci, A. S. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 4167–4171.
11. Fava, R. A., Olsen, N. J., Postlethwaite, A. E., Broadley, K. N., Davidson, J. M., Nanney, L. B., Lucas, C. & Townes, A. S. (1991) *J. Exp. Med.* **173,** 1121–1132.
12. Akiyama, S. K., Nagata, K. & Yamada, K. M. (1990) *Biochim. Biophys. Acta* **1031,** 91–110.
13. Hynes, R. O. (1987) *Cell* **48,** 549–554.
14. Assoian, R. K., Komoriya, A., Meyers, C. A., Miller, D. M. & Sporn, M. B. (1983) *J. Biol. Chem.* **258,** 7155–7160.
15. Assoian, R. K., Fleurdelys, B. E., Stevenson, H. C., Miller, P. J., Madtes, D. K., Raines, E. W., Ross, R. & Sporn, M. B. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 6020–6024.
16. McCartney-Francis, N., Mizel, D., Wong, H., Wahl, L. M. & Wahl, S. M. (1990) *Growth Factors* **4,** 27–35.
17. Kerhl, J. H., Wakefield, L. M., Roberts, A. B., Jakowlew, S. B., Alvarez-Mon, M., Derynck, R., Sporn, M. B. & Fauci, A. B. (1986) *J. Exp. Med.* **163,** 1037–1050.
18. Collier, I. E., Wilhelm, S. M., Eisen, A. Z., Marmer, B. L., Rant, G. A., Seltzer, J. L., Kronberger, A., He, C., Bauer, E. A. & Goldberg, G. I. (1988) *J. Biol. Chem.* **263,** 6579–6587.
19. Wilhelm, S. M., Collier, I. E., Marmer, B. L., Eisen, A. Z., Grant, G. A. & Goldberg, G. I. (1989) *J. Biol. Chem.* **264,** 17213–17221.
20. Wahl, L. M., Katona, I. M., Wilder, R. L., Winter, C. C., Haraoui, B., Scher, I. & Wahl, S. M. (1984) *Cell Immunol.* **85,** 373–383.
21. Akiyama, S. K., Yomada, S. S., Chen, W. T. & Yamada, K. M. (1989) *J. Cell Biol.* **109,** 863–875.
22. Klebe, R. J. (1974) *Nature (London)* **250,** 248–251.
23. Miekka, S. I., Inghaam, K. C. & Menache, D. (1982) *Thromb. Res.* **27,** 1–14.
24. Timpl, R., Rhode, A., Gehron, P. G., Rennard, S. I., Foidart, J. M. & Martin, G. R. (1979) *J. Biol. Chem.* **254,** 9933–9937.
25. Kleinman, H. K., McGarvey, M. L., Liotta, L. A., Robey, P. G., Tryggvason, K. & Martin, G. (1982) *Biochemistry* **24,** 6188–6193.
26. Humphries, M. J., Komoriya, A., Akiyama, S. K., Olden, K. & Yamada, K. M. (1987) *J. Biol. Chem.* **262,** 6886–6892.
27. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–161.
28. Argraves, W. C., Suzuki, S., Arai, H., Thompson, K., Pierschbacher, M. D. & Ruoslahti, E. (1987) *J. Cell Biol.* **105,** 1183–1190.
29. Reed, J. C., Alpers, J. D. & Nowell, P. G. (1987) *J. Clin. Invest.* **80,** 101–106.
30. Brown, D. L., Phillips, D. R., Damsky, C. H. & Charo, I. F. (1989) *J. Clin. Invest.* **84,** 366–370.
31. Roth, P. & Polin, R. A. (1990) *Clin. Immunol.* **57,** 363–373.
32. Shaw, L. M. & Mercurio, A. M. (1989) *J. Exp. Med.* **169,** 303–308.
33. Roberts, C. J., Birkenmeier, T. M., Mcquillan, J. J., Akiyama, S. K., Yamada, S., Chen, W. T., Yamada, K. M. & McDonald, J. A. (1988) *J. Biol. Chem.* **263,** 4586–4592.
34. Heino, J., Ignotz, R. A., Hemler, M. E., Crouse, C. & Massagué, J. (1989) *J. Biol. Chem.* **264,** 380–388.
35. Ignotz, R. A., Heino, J. & Massagué, J. (1989) *J. Biol. Chem.* **264,** 389–392.
36. Shimizu, Y. & Shaw, S. (1991) *FASEB J.* **5,** 2292–2299.
37. Krissansen, G. W., Elliott, M. J., Lucas, C. M., Stomski, F. C., Berndt, M. C., Cheresh, D. A., Lopez, A. F. & Burns, G. F. (1990) *J. Biol. Chem.* **265,** 823–830.
38. Andreesen, R. A., Brugger, W., Scheibenbogen, C., Kreutz, M., Leser, H. G., Rehm, A. & Lohr, G. W. (1990) *J. Leuk. Biol.* **47,** 490–497.
39. Wahl, L. M., Corcoran, M. L., Mergenhagen, S. E. & Finbloom, D. S. (1990) *J. Immunol.* **144,** 3518–3522.
40. Welgus, H. G., Campbell, E. J., Cury, J. D., Eisen, A. Z., Senior, R. M., Wilheim, S. M. & Goldberg, G. I. (1990) *J. Clin. Invest.* **86,** 1496–1502.
41. Garbisa, S., Ballin, M., Daga-Gordini, D., Fastelli, G., Naturale, M., Negro, A., Semenzato, G. & Liotta, L. A. (1986) *J. Biol. Chem.* **261,** 2369–2375.
42. Overall, C. M., Wrana, J. L. & Sodek, J. (1989) *J. Biol. Chem.* **264,** 1860–1869.
43. Edwards, D. R., Murphy, G., Reynolds, J. J., Whitman, S. E., Doherty, A. J. P., Angel, P. & Heath, J. K. (1987) *EMBO J.* **6,** 1899–1904.
44. Salo, T., Lyons, J. G., Rahemtulla, F., Birkedal-Hansen, H. & Larjava, H. (1991) *J. Biol. Chem.* **266,** 11426–11441.
45. Wahl, S. M. (1992) *J. Clin. Immunol.* **12,** 61–74.
46. Wahl, S. M., Allen, J. B., Costa, G. L., Wong, H. L. & Dasch, J. R. (1993) *J. Exp. Med.* **177,** 225–230.