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Inflammation Research

Fibronectin regulates the activation of THP-1 cells by TGF-β1

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Abstract. *Objective and Design:* To determine how fibronectin regulates the immunomodulatory effects of transforming growth factor (TGF)-β on THP-1 cells.
Material or Subjects: THP-1 monocytic cell line.
Treatment: THP-1 cells were primed for 48 h in the presence or absence of 250 pM TGF-β1.
Methods: Assays or assessments carried out, together with statistical test applied.
Results: We found that adherence to fibronectin dramatically modulates the effects of TGF-β1 on the human monocytic cell line THP-1. TGF-β1 did not significantly affect constitutive interleukin (IL)-8 secretion or IL-1β-induced IL-8 secretion from suspended cells. In contrast, TGF-β1 stimulated IL-8 secretion as well as augmented IL-1β-induced IL-8 secretion from adherent cells. The differential effects of TGF-β1 on IL-8 secretion from suspended and adherent cells could not be explained by differences in IL-1 receptor antagonist production. The effects of fibronectin on TGF-β1 induced IL-8 secretion from THP-1 cells were mimicked by adhesion to immobilized anti-α4β1 integrin antibody and to a fibronectin fragment containing the CS-1 domain.
Conclusions: These results indicate that α4β1-mediated adhesion to fibronectin may play a key role during inflammation by profoundly influencing the effects of TGF-β1 on monocytes.

Key words: Monocytes/macrophages – Inflammation – Adhesion molecules – Inflammatory mediators – Growth factors

Introduction

Monocytes play a key role in the initiation of inflammation. In response to injurious agents such as lipopolysaccharide (LPS), monocytes secrete "early response" cytokines such as such as interleukin (IL)-1β that then trigger the activation of other macrophages and resident tissue cells such as endothelial cells, epithelial cells, and fibroblasts. In particular, IL-1β induces the secretion of IL-8, a potent neutrophil chemoattractant. Precise regulation of monocyte activation and IL-8 expression is essential to avoid excessive inflammatory cell recruitment.

Transforming growth factor (TGF)-β1 is a potent immunomodulatory molecule likely to be encountered by monocytes at inflammatory sites. In vitro and in vivo data suggest that TGF-β1 can suppress as well as promote inflammation [1–7]. The pro- or anti-inflammatory activities of TGF-β1 appear to be regulated by factors present within the local microenvironment. While locally administered TGF-β1 predominantly acts to recruit and activate leukocytes [8], systemic administration enables TGF-β1 to exert primarily immunosuppressive effects [9]. For instance, direct injection of TGF-β1 into the joint spaces of rats resulted in a synovitis characterized by monocyte infiltration and activation [8]. In this study, TGF-β1 was found to directly promote monocyte recruitment and chemotaxis and also stimulated IL-1β expression. In contrast, systemically administered TGF-β1 suppressed the development of inflammation in an experimental model of acute and chronic arthritis [5]. One possible explanation for these findings is that extracellular matrix-mediated signals modulate the effects of TGF-β1 on target cells.

During inflammation, peripheral blood monocytes migrate into inflamed tissues and are activated. Adherence to the extracellular matrix plays a key role in mediating this activation process by direct effects on inflammatory gene expression as well as by facilitating the effects of cytokines and growth factors present within the local milieu. Injured tissues are particularly rich in the extracellular matrix protein fibronectin (Fn). Evidence suggests that Fn induces the expression of several pro-inflammatory cytokines, including IL-1β, TNF-α and IL-8, in monocytes [10, 11]. In addition, cell-matrix interactions and IL-1-dependent signaling interact to regulate mesenchymal cell function by stimulating nuclear factor-κB (NF-κB) activity and inducing IL-6 gene expression [12–14]. Fn can also regulate the action of soluble growth factors. In a recent study by Lin, et al, EGF treatment of adherent cells resulted in much stronger activations of Raf-B, MEK and MAP kinase as compared with non-

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adherent cells. Similarly, activation of Raf-1 and MEK was far greater in anchored cells following PDGF stimulation than in nonanchored cells [15].

We investigated the role of cell-matrix interactions in regulating the effects of soluble mediators on monocytes by examining the effects of Fn on IL-1β- and TGF-β1-stimulated IL-8 secretion. For these studies, we used THP-1 cells, a human monocyte-like cell line that mimics several crucial monocyte responses to growth factors and extracellular matrix proteins. In addition, the integrin repertoire of THP-1 cells is similar to that of peripheral blood monocytes [16]. We provide evidence that the induction of IL-8 secretion by IL-1β and TGF-β1 is critically influenced by whether or not cells are adherent to Fn. Furthermore, our findings suggest a biologically significant model in which the integrin α4β1 potentiates the pro-inflammatory effects of TGF-β1 on monocytes during inflammation.

Materials and methods

Materials

Monoclonal antibodies against α5β1 (P3D10), αvβ5 (P1F6), αvβ6 (R6G9) and vitronectin were a generous gift of Dr. Dean Sheppard (Lung Biology Center, San Francisco, CA). The anti-α4β1 antibody (P4C2) was obtained from Pharmingen (La Jolla, CA). Anti-α2β1 (P1E6), anti-α3β1 (P1B3) and human laminin were obtained from Life Technologies, Inc (Gaithersburg, MD). In some experiments, a second anti-α5β1 antibody (catalog number 05-230, Upstate Biotechnology, Saranac Lake, NY) was used. Human fibronectin was obtained from Calbiochem (La Jolla, CA). Recombinant fibronectin fragments containing the COOH-terminal domain with the CS-1 sequence (Fn 9.5) were expressed and purified as a GST-fusion protein from bacteria transformed with a GST-fusion vector PGEX2T/BL21DE3 (generous gift of Dr. James B. McCarthy, University of Minnesota, Minneapolis, MN, [17]). Briefly, bacteria were cultured overnight in the presence of 50 µg/ml ampicillin. IPTG, 0.1 mM was added for 2 h at 37 °C. The bacteria were spun down and lysed, then sonicated. After the addition of DNaseI, glutathione sepharose 4B beads (Amersham Pharmacia Biotech, Piscataway, NJ) were then added and the protein purified according to manufacturer's protocol.

Cell Culture

THP-1 cells were maintained in RPMI 1640 medium containing 10% fetal calf serum (heat inactivated), 100 µg/ml streptomycin and 100 units/ml penicillin. For experiments, PBMC or THP-1 cells were maintained in suspension culture on teflon-coated plates (Corning-Costar, Acton, MA) in 2% FBS ± 250 pM TGFβ1 for 48 h before culturing them on coated dishes in the presence or absence of IL-1β (Fig. 1). Substratum-coated dishes were prepared by incubating stated concentrations of fibronectin or fibronectin fragment, poly-L-lysine, anti-α4β1 or anti-α5β1 antibodies in phosphate-buffered saline (PBS) in tissue culture dishes at 4 °C overnight. The dishes were blocked with 0.1% bovine serum albumin (BSA) and washed with PBS prior to use. For integrin engagement studies, THP-1 cells were suspended in RPMI/2% FCS at 5×10^6 cells/ml.

Primary blood monocytes (PBMC) were harvested from volunteers by leukapheresis. Mononuclear cells were purified by Percoll sedimentation and monocytes separated from lymphocytes by adherence to 2% gelatin. Adherent cells were removed with 5 mM EDTA/ 5% serum, washed and resuspended in RPMI 1640/10%HIHS (heat inactivated human serum).

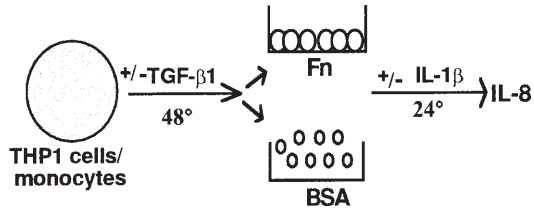

Fig. 1. Schematic of experimental protocol. THP-1 cells or monocytes were conditioned with or without 250 pM TGF-β1 for 48 h and then transferred to tissue culture plates coated with either BSA (maintained in suspension culture) or allowed to adhere to Fn (10 µg/ml). Cells were then treated with or without IL-1β (10 ng/ml). Conditioned media were harvested after 24 h and IL-8 secretion assessed by ELISA.

IL-8 and IL-1ra ELISAs

Ninety-six-well medium-binding plates (Corning-Costar, Acton, MA) were coated with a monoclonal goat anti-human IL-8 antibody (R & D Systems, Minneapolis, MN) overnight at 4 °C and blocked with 0.1% BSA for 1–2 h at 25 °C (RT). Equal aliquots of conditioned media were harvested and placed into wells. In some cases, the media was diluted up to 1 : 50 to insure that the concentration of IL-8 was within the range of the assay. After 2–2.5 h incubation at RT, media was removed, wells were washed with PBS/0.05% Tween and then incubated for 1.5 h with a polyclonal rabbit anti-human IL-8 antibody (Upstate Biotechnology, Saranac Lake, NY). The wells were washed and biotinylated goat anti-rabbit secondary antibody applied (Vector, Burlingame, CA). After washing, wells were incubated with streptavidin alkaline phosphatase (Jackson Immunoresearch, West Grove, PA) and detection of captured IL-8 complexes achieved using an alkaline phosphatase substrate kit (Sigma, St. Louis, MO). Absorbance was measured at 405 nm using a Microplate Devices reader. In parallel experiments, conditioned media were harvested as described and IL-1ra secretion assessed using an IL-1ra ELISA kit (R & D Sys, Minneapolis, MN) according to the manufacturer's directions.

Adhesion Assay

Adhesion studies were performed by coating 96-well tissue culture plates (Corning-Costar, Acton MA) for overnight at 4 °C. Wells were blocked with BSA (0.1% in PBS) for 30 min^{-1} h at 37 °C. Control wells were coated with BSA alone. All wells were washed with PBS prior to use. THP-1 cells were washed, resuspended in RPMI 1640 medium with or without anti-integrin antibodies for 20 min on ice and added to coated wells (5×10^4 cells/well) for 1 h at 37 °C. The wells were then washed twice with PBS and stained with 0.1% crystal violet/10% methanol in PBS for 15 min. Cells were then washed three more times with PBS and the crystal violet stain solubilized with 2% triton/PBS. Absorbance was measured at 595 nm. Each assay was done in triplicate.

Flow cytometry

Cells were blocked with normal goat serum (Vector, Burlingame, CA) for 10 min. We found in previous experiments that this procedure did not affect detection of any of the integrins examined compared to removal using EDTA alone. Cells were then incubated with the appropriate primary antibody (see below) for 20 min, washed with PBS and stained with secondary goat anti-mouse IgG conjugated to phycoerythrin (Chemicon, Temecula, Ca). Surface integrin expression was analyzed by FACScan (Becton Dickinson, San Jose, CA).

Statistical analyses

All data are expressed as mean ± sem for a series of n conditions. Analysis of variance (ANOVA) with the Student-Newman-Keuls post-test

were used to compare mean values as appropriate. P values < 0.05 were considered to represent significant differences.

Results

TGF-β1 stimulates IL-8 secretion from adherent, but not suspended THP-1 cells

Suspended THP-1 cells secreted very little IL-8 at baseline (Fig. 2A). As expected, adherence to Fn upregulated IL-8 secretion (Fig. 2B), while the combination of IL-1β and Fn had an additive effect on IL-8 secretion. TGF-β1 pretreatment did not significantly affect constitutive or IL-1β-stimulated IL-8 secretion from suspended cells. In contrast, TGF-β1-priming significantly stimulated IL-8 secretion from adherent cells and also potentiated IL-1β-stimulated IL-8 secretion from adherent cells. Adherent, TGF-β1-treated cells secreted approximately 20-fold more IL-8 than TGF-β1 treated suspended cells. Together, IL-1β and TGF-β1 stimulated nearly 10-fold more IL-8 secretion from adherent cells compared to suspended cells. To confirm these results, primary monocytes were primed with TGF-β1 for 48 h and then stimulated with IL-1β for 24 h under adherent or nonadherent conditions. As shown in figure 2C, adherence to Fn significantly upregulates IL-8 secretion from TGF-β1 primed primary blood monocytes.

In order to determine whether the effects of Fn were due to nonspecific effects of adherence, cells were pretreated with TGF-β1 as described and then incubated in tissue culture plates coated with BSA, Fn, poly-L-lysine. As shown in Fig. 3, adhesion to poly-L-lysine failed to significantly stimulate IL-8 secretion from THP-1 cells (Fig. 3).

The integrins α4β1 and α5β1 are the principal fibronectin receptors on THP-1 cells.

THP-1 cells express at least two Fn receptors: α4β1 and α5β1. Whether THP-1 cells express other potential Fn receptors such as α4β7 and αvβ5 has not been resolved [16, 18]. We therefore surveyed the surface expression of integrins on

Table 1. Integrins constitutively expressed by THP-1 cells.[a]

(potential) fibronectin receptors	Mean fluorescent units
α4β1	42.8
α5β1	16.5
αvβ5	7.3
α3β1	6.2
α4β7	3.4
collagen-laminin receptors	7.2
α2β1	3.5
α6β1	3.5
(control, no 1° Ab)	3.4

[a] THP-1 cells were incubated with a panel of mouse monoclonal-anti-human integrin antibodies, washed and then stained with phycoerythrin conjugated goat anti-mouse antibody. Surface integrin levels were measured by flow cytometry. No significant surface levels of α6β1, αvβ3, α4β7, or αvβ6 were detected. Data are presented as mean fluorescent units of a representative experiment.

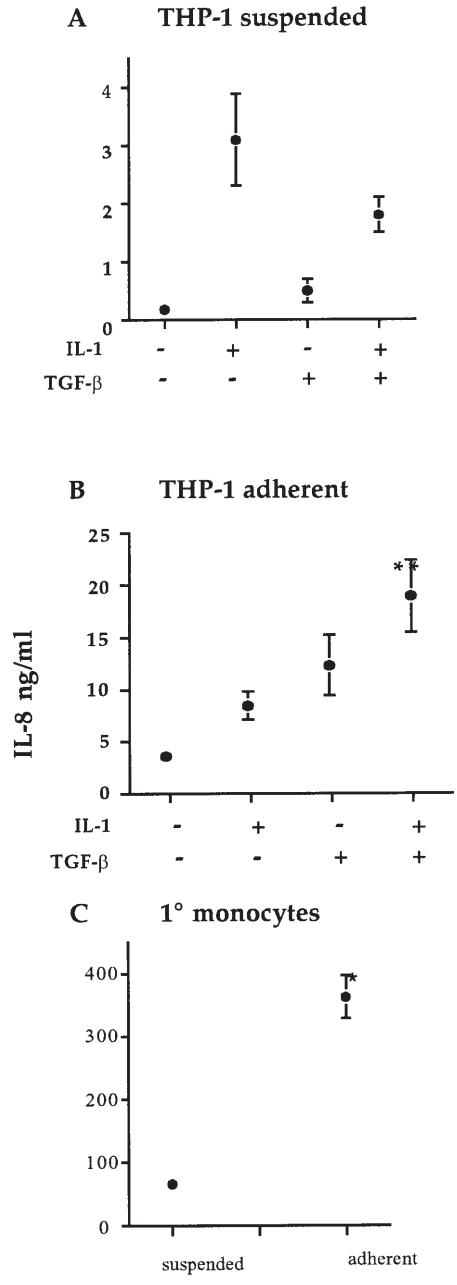

Fig. 2. Fn enhances IL-1β and TGF-β1 stimulated IL-8 secretion from THP-1 cells and primary monocytes. Suspended THP-1 cells were cultured in the absence or presence of TGF-β1 for 48 h. Then, equal number of cells were transferred to tissue culture plates coated with (**A**) BSA (suspension culture) or (**B**) Fn (10 µg/ml) and stimulated with or without IL-1β (10 ng/ml). After 24 h, IL-8 concentrations in equal aliquots of conditioned media were assayed by ELISA. Data are expressed as mean ± SEM of 5 independent experiments. All conditions were performed in triplicate. * indicates significant difference in IL-8 secretion from TGF-β1-primed, adherent cells versus TGF-β1-primed, suspended cells ($p < 0.001$) and from unstimulated, adherent cells. ** indicates significant difference in TGF-β1-primed, IL-1β-stimulated *adherent* cells versus TGF-β1-primed, IL-1β-stimulated *suspended* cells ($p < 0.001$). (**C**) Suspended monocytes were cultured in the presence of TGF-β1 for 48 h as described. Then, equal number of cells were transferred to tissue culture plates coated with BSA (suspended) or Fn (adherent) and stimulated with IL-1β for 24 h. IL-8 secretion was assessed by ELISA. Data are expressed as mean ± SEM of two independent experiments. ***indicates significant difference between TGF-β1-primed *adherent* cells versus TGF-β1-primed *suspended* cells ($p < 0.001$).

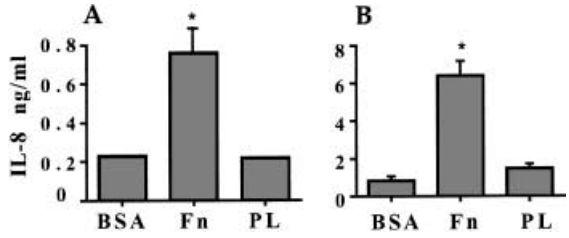

Fig. 3. poly-L-lysine does not stimulate IL-8 secretion from THP-1 cells. Suspended cells were cultured in the (**A**) absence or (**B**) presence of TGF-β1 for 48 h. Then equal numbers of cells were transferred to plates coated with BSA, fibronectin (Fn) or poly-L-lysine (PL). *All* dishes were treated with IL-1β for 24 h and IL-8 secretion assessed by ELISA. All conditions were performed in triplicate. (Representative experiment of 3). Data are presented as mean ± SEM of two independent experiments. *$p < 0.05$ compared to cells maintained in suspension culture or adherent to poly-L-lysine. Error bars not shown were too small to be graphed.

THP-1 cells using flow cytometry (Table 1). We found that in addition to α4β1 and α5β1, THP-1 cells maintained in suspension culture express low levels of two other potential Fn receptors, α3β1 and αvβ5. They do not express significant levels of αvβ3, α4β7 or αvβ6. Consistent with previous reports, suspended THP-1 cells also express the collagen/laminin receptor α2β1. We did not detect significant levels of α6β1, however.

Next, we used a panel of anti-integrin monoclonal antibodies to determine which of the potential Fn receptors were actually involved in mediating adhesion to Fn (Fig. 4). Anti-α5β1 antibody (P3D10) alone decreased attachment by approximately 70%. Although anti-α4β1 antibody (P4C2) has been previously shown to block attachment of hematopoetic cells and T-lymphocytes to fibronectin [19], it had no effect on cell attachment of THP-1 cells when used alone. However, the combination of monoclonal anti-α4β1 and anti-α5β1 antibodies consistently inhibited adhesion by approximately 90%. In contrast, the addition of either anti-αvβ5 or anti-αv antibodies did not affect cell adhesion either when used alone or in combination with the anti-α5β1 antibody (data not shown). Hence, although THP-1 cells express far fewer α5β1 than α4β1 on their surface, α5β1 appears to be the principal integrin mediating adhesion to Fn.

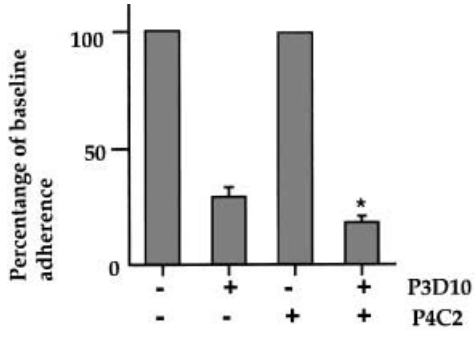

Fig. 4. α4β1 and α5β1 are the principal fibronectin receptors on THP-1 cells. THP-1 cells were allowed to adhere to Fn-coated 96-well plates in the presence or absence of anti-integrin antibodies. Data are expressed as percentage of baseline adherence (untreated cells) compared with cells incubated with anti-α5β1 antibody (P3D10, 10 µg/ml), anti-α4β1 antibody (P4C2, 10 µg/ml) or both antibodies in combination (mean ± SEM of 3 experiments). Each condition was assessed in triplicate. *indicates significant difference in adhesion ($p < 0.005$) compared to using anti-α5β1 antibody alone.

Adhesion to immobilized anti-α4β1 antibody and COOH-terminal CS-1 containing Fn fragment (F9.5) augments TGF-β1 induced IL-8 secretion

We then examined the potential roles of α4β1 and α5β1 in modulating the effects of TGF-β1 on THP-1 cells. Tissue culture dishes were coated with BSA, anti-α5β1 antibody (P3D10), or an antibody directed against α4 (P4C2). THP-1 cells were treated with or without TGF-β1 and then allowed to adhere to coated dishes. Adhesion to surfaces coated with either anti-α5β1 or anti-α4β1 antibody resulted in enhanced IL-8 secretion from non-treated cells (Fig. 5A). TGF-β1 did not significantly affect IL-8 secretion from cells attached to immobilized anti-α5β1 antibody (Fig. 5B). Similar results were obtained when cells were allowed to adhere to plates coated with another anti-α5β1 antibody. In contrast, TGF-β1 primed cells adherent on anti-α4 antibody secreted approximately 5-fold more IL-8 than non-treated cells. This results strongly suggest that the α4β1 integrin is involved in mediating the effects of Fn on TGF-β1-induced IL-8 secretion.

α4β1 binds to the alternatively spliced CS-1 sequence contained within the carboxy-terminal portion of Fn. To further assess the role of α4β1 in mediating the effects of TGF-β1 on monocytes, we investigated whether adherence to a recombinant Fn fragment containing the CS-1 region was sufficient for TGF-β1 to stimulate IL-8 secretion from THP-1 cells. This fragment (F9.5) does not contain the central cell binding domain that interacts with the α5β1 integrin. As shown in figure 6, TGF-β1 stimulated IL-8 secretion from THP-1 cells plated on the F9.5 fragment, but not from suspended cells. These data strongly suggest that the integrin α4β1 is involved in augmenting the effects of TGF-β1 on IL-8 secretion from THP-1 cells.

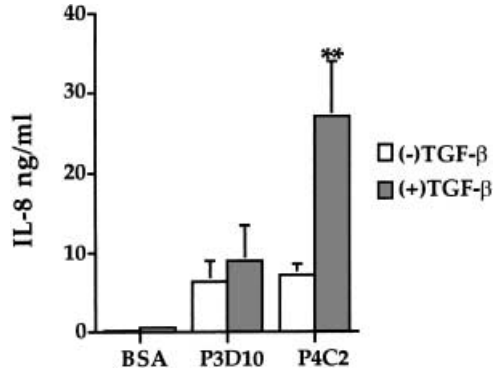

Fig. 5. Adhesion to immobilized anti-α4β1 antibody enhances IL-1β and TGF-β1 stimulated IL-8 secretion from THP-1 cells. IL-8 secretion from THP-1 cells cultured on immobilized anti-integrin antibodies. THP-1 cells were pretreated with or without TGF-β1 for 48 h and then cultured in 6-well plates coated with either BSA, anti-α5β1 antibody (P3D10, 10 µg/ml), or anti-α4 antibody (P4C2, 10 µg/ml). The antibodies are of the same isotype. Data are expressed as mean ± SEM of 4 independent experiments. All conditions were performed in triplicate. ** indicates significant difference in IL-8 secretion between TGF-β1 treated and untreated cells adherent on P4C2 ($p < 0.005$).

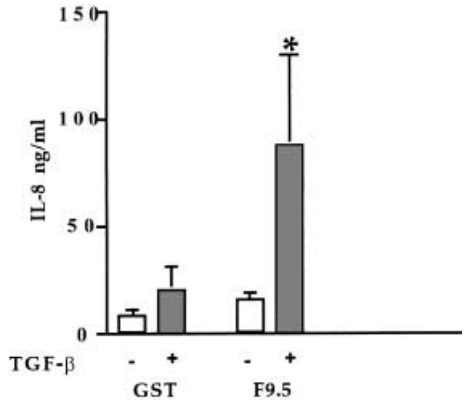

Fig. 6. Adhesion to CS-1 containing Fn fragment (F9.5) enhances TGF-β1 stimulated IL-8 secretion from THP-1 cells. THP-1 cells were pretreated without or with TGF-β1 for 48 h and then cultured in 6-well plates coated with either GST (GST, 10 µg/ml) or a GST-Fn fragment containing the CS-1 sequence, but not RGD (F9.5, 10 µg/ml). The concentration of secreted IL-8 in equal aliquots of conditioned media was measured by ELISA. Data are expressed as mean ± SEM of 3 independent experiments. All conditions were performed in triplicate. *indicates significant difference ($p < 0.001$) in IL-8 secretion compared to either suspended cells treated with TGF-β1 or adherent cells that were not treated with TGF-β1.

Role of IL1 receptor antagonist

The IL-1 receptor antagonist is 20–30% homologous with IL-1α and IL-β and binds to both the 80 kDa type I and 67 kDa type II IL-1 receptors in humans. Thus, it functionally blocks IL-1 activity. TGF-β1 was previously reported to inhibit IL-1β-induced IL-8 secretion from suspended monocytes [7]. In that study, TGF-β1 initially stimulated IL-1β secretion, but also induced expression of the IL-1 receptor antagonist (IL-1ra) through an IL-1 dependent mechanism. Based on these data, we sought to determine whether the different responses of adherent and suspended cells to TGF-β1 pretreatment could be accounted for by differences in IL-1ra

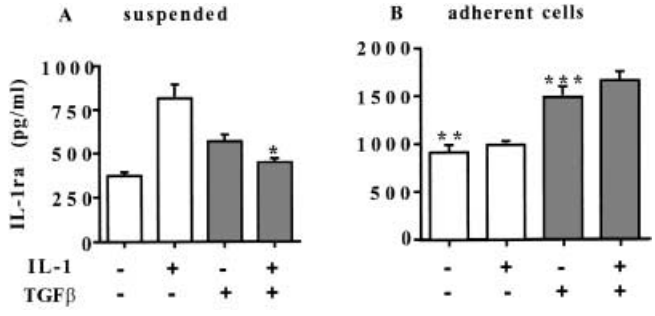

Fig. 7. Effects of Fn and TGF-β1 on IL-1ra secretion. Cells were treated with TGF-β1 as described and allowed to remain in suspension or adhere to Fn-coated tissue culture plates in the absence or presence of IL-1β. After 24 h, secretion of IL-1ra was assessed by ELISA. All conditions were performed in triplicate. Data are expressed as mean ± SEM. (Representative experiment of 3). * indicates significant difference compared to cells stimulated with IL-1β alone ($p < 0.001$) **indicates significant difference compared to cells maintained in suspension ($p < 0.001$), ***indicates significant difference compared to cells stimulated by adherence alone ($p < 0.001$)

production. As depicted in Fig. 7A, while TGF-β1 did increase IL-1ra secretion from suspended cells, it actually inhibited IL-1β-induced secretion of IL-1ra from suspended cells. In contrast, TGF-β1 stimulated IL-1ra secretion from adherent cells (Fig. 7B). Thus, the differential effects of TGF-β1 on IL-8 secretion from suspended and adherent cells cannot be entirely explained through its effects on IL-1ra production.

Discussion

Since monocytes are likely to encounter pro-inflammatory stimuli before they leave the bloodstream, mechanisms must exist that restrict inflammatory protein expression to cells present within sites of injury. A logical means of accomplishing this is through the extracellular matrix. In our study, Fn not only augmented IL-1β-induced IL-8 secretion from THP-1 cells but also profoundly affected the ability of TGF-β1 to activate THP-1 cells. While TGF-β1 priming upregulated IL-8 secretion from adherent cells it had no significant effect on suspended cells. Furthermore, while TGF-β1 *inhibited* IL-1β-induced IL-8 secretion from suspended cells, it *potentiated* IL-1β -stimulated IL-8 secretion from cells adherent on Fn. To our knowledge, our findings are the first to demonstrate a possible mechanism through which the pro- and anti-inflammatory effects of TGF-β1 can be regulated and suggest that the adherence-dependent effects of TGF-β1 on THP-1 cells are mediated at least in part by the integrin α4β1.

Lin, et al. [11] have demonstrated that Fn induces cytokine gene expression in THP-1 cells. THP-1 cells express multiple potential Fn receptors; however, in vitro adhesion assays suggest that only α4β1 and α5β1 are involved in mediating adhesion to Fn. α4β1 and α5β1 interact with different sites within the Fn molecule. α5β1 interacts with the RGD sequence in the central cell binding domain of Fn while α4β1 binds the CS-1 sequence located in the carboxy terminal end of Fn. Recent data suggest that the α cytoplasmic domains of various integrins can specifically activate distinct signaling pathways [20]. Thus, integrins can have different effects of cellular gene expression despite binding to the same extracellular matrix ligand. While α4β1 does not appear to play a major role in mediating cell adherence to Fn, our findings suggest that it is capable of mediating the adherence-dependent effects of TGF-β1. THP-1 cells express at least two subsets of α4β1 on their cell surface: one composed of transiently active or low affinity receptors and an inactive pool [21]. TGF-β1 does not increase THP-1 cell surface expression of α4β1 (data not shown, [16]). Whether TGF-β1 may be modulating the interaction of α4β1 with Fn by increasing the number of receptors available for ligand binding or by regulating the affinity of already active receptors is the subject of ongoing studies.

One potential mechanism through which TGF-β1 and Fn could regulate IL-8 expression is by modulating expression of the IL-1 receptor antagonist IL-1ra. TGF-β1 has been previously shown to stimulate IL-1ra secretion from suspended monocytes through an IL-1 dependent pathway. [7]. However, both TGF-β1 and Fn increased IL-1ra secretion. In addition, although TGF-β1 pretreatment increased IL-1ra

secretion from suspended THP-1 cells, TGF-β1 primed suspended cells actually secreted *less* IL-1ra following IL-1β stimulation than cells that had not been pretreated. Hence, the effects of TGF-β1 and Fn on IL-8 expression in THP-1 cells cannot be explained through their effects on IL-1ra expression.

The fact that a priming period of between 24–48 h was required raises the possibility that TGF-β1 priming may result in the synthesis of signaling intermediate(s) that act in conjunction with adherence induced signaling cascades to promote IL-8 gene transcription or enhance IL-8 mRNA stability [22]. A growing number of studies demonstrate that integrin- and cytokine/growth factor-transduced signal transduction pathways overlap at several levels [23, 24]. In addition to modulating the effects of TGF-β1, adherence to Fn increased IL-8 secretion from IL-1β-stimulated THP-1 cells. In contrast, Fn failed to enhance IL-1β-stimulated IL-1ra secretion. Our finding that Fn differentially modulates the effects of IL-1β on IL-8 and IL-1ra secretion suggests that adherence is not merely increasing the number or function of surface IL-1 receptors [25] but is facilitating the activation of specific signaling pathways. Since transcription of IL-8 and IL-1ra are both regulated through NF-κB, another transcription factor may be involved in mediating the distinct effects of Fn on IL-1β dependent gene regulation. For instance, IL-8 gene transcription also depends on AP-1. In rabbit synovial fibroblasts, While AP-1 has been reported to regulate IL-8 gene transcription [26, 27] its role in regulating IL-8 expression in monocytes is remains to be defined.

In summary, our findings suggest a potential mechanism through which the onset and extent of inflammation can be controlled in vivo. In this model, TGF-β1 initially acts to suppress the activation of circulating cells. TGF-β1 not only directly activates but also "primes" cells that are adherent at sites of injury to respond maximally to pro-inflammatory cytokines such as IL-1β. These findings are consistent with a previous study in which injection of a Fn fragment containing the CS-1 domain blocked the development of acute and chronic synovial inflammation in an experimental model of arthritis [28]. The CS-1 peptide suppressed the development of chronic inflammatory changes even when injected 11 days following the onset of acute arthritis. In addition, the efficacy of anti-α4 mAb treatment in a murine model of asthma has been linked to parenchymal α4-expressing cells [29]. Like THP-1 cells, primary blood monocytes also express the α4β1 integrin. Leukocyte integrins such as α4β1 clearly play a key role in mediating attachment and migration from the vasculature [30, 31]. Their adhesive interactions within peripheral tissues may play an equally important role in inflammation. Hence, the use of anti-adhesion therapy in treating inflammation must not only consider the target of action, but also the timing and site of delivery as well.

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