Interleukin-1β expression is induced by adherence and is enhanced by Fc-receptor binding to immune complex in THP-1 cells

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Abstract Adherence of monocytes to endothelial cells and subsequently to basement membrane represents the initial steps in monocyte migration from the vasculature to the interstitium. We investigated the role of adhesion to endothelial cells and basement membrane in the induction of the cytokine IL-1β. We demonstrated that mRNA for IL-1β is induced in adherent THP-1 cells, but not in a matrix-specific manner. Adherence to fibrinogen, however, causes an increase in mRNA for IL-1β. A background level of IL-1β mRNA induction was observed in cells adherent to all matrices, including the non-specific human serum albumin substrate, as compared to non-adherent cells cultured in teflon troughs. In addition, antibodies to CD11a, CD11b, β_1 integrin, VLA4, αvβ3 (VNR), and ICAM-1 did not induce significant IL-1β mRNA when THP-1 cells were adherent to those immunoglobulins. THP-1 cells adherent to immune complexes of anti-CD11a, anti-CD11b, anti-VLA4, anti-VNR, and anti-ICAM-1 showed greater mRNA induction than cells adherent to primary antibodies alone. THP-1 cells adherent to non-specific immune complexes gave the highest level of mRNA induction. Secretion of IL-1β protein, measured by ELISA at 24 h, was greatest when cells were adherent to immobilized immune complexes or to fibrinogen. Our results demonstrate that a general adherence-induced increase in IL-1β gene expression is greatly enhanced by the presence of immune complex.

Key words: Interleukin-1β; Monocyte; Adherence; Immune complex

1. Introduction

Monocytes are recruited to sites of inflammation and infection. During their emigration from the peripheral blood, they interact with adhesion receptors on the endothelial cells and ultimately come into contact with extracellular matrix proteins. Monocytes express a variety of integrins, receptors for extracellular matrix proteins. Interactions of these integrins with their ligands are known to have major effects on monocyte function and maturation including stimulation of secretion of cytokines, cytotoxicity, and gene expression [1–6]. Among the extracellular matrix proteins that interact with monocyte adhesion receptors are fibronectin, vitronectin and collagen. Monocytes interact with these matrix components through members of the β1 and β3 integrin families.

Monocytes also recognize and adhere to cell surface molecules on endothelial cells lining capillaries and venules, including the counter receptors intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1). These cell surface proteins are cytokine inducible and are known to be involved in the emigration of immune cells. Monocytes interact with VCAM-1 through the α4β1 (VLA4) cell surface receptor [7], with fibronectin through α5β1 and VLA4, and with ICAM-1 and fibrinogen through Mac-1 [8,9]. These interactions with the endothelial cell may also result in altered gene expression and further stimulation of the monocyte [5].

In addition, monocytes express FcRγI, FcRγII and FcRγIII, receptors for IgG. These receptors transduce signals resulting in tyrosine phosphorylation of $p72^{syk}$, the Fc receptor gamma chain and paxillin, and the induction of cytokine gene expression [10,11]. Previous studies have shown that cross-linking the Fc receptors on monocytes will induce production of tumor necrosis factor-α (TNF-α) and interleukin-6 [11,12]. A recent study observed activity of Mac-1 in polymorphonuclear cells after engagement of FcγRII resulting in tyrosine phosphorylation [13], suggesting that signals transduced by Mac-1 and FcγRII in monocytes may also be coordinated.

Adherence of monocytes/macrophages to specific substrates results in transcriptional upregulation of a variety of genes [4,5,14–17]. In addition, Yurochko et al. [18] demonstrated that direct engagement of the β1 integrin, but not the β2 integrin, by antibody crosslinking of the integrin receptor results in the upregulation of several immediate early genes. Among the immediate early genes that are induced by adherence and/or cross-linking with antibodies are interleukin-1β (IL-1β), TNF-α, interleukin-8 (IL-8), c-myc, c-myb, and the inhibitor of kappa-B [19]. Lin et al. [20] showed that tyrosine phosphorylation of a 76 kDa protein coincided with adherence-induced IL-1β message induction in human monocytes.

In the present study we tested the hypothesis that engagement of the integrin receptors by adherence to their natural ligands would result in the transcriptional upregulation of IL-1β in the monocytic cell line, THP-1 cells. In addition, we investigated the possibility that the signals transmitted after engagement of the immunoglobin receptor, FcRγI or FcRγII, by IgG or immune complexes would induce IL-1β gene expression when cells are adherent.

2. Materials and methods

2.1. RNA isolation and PCR

Total RNA was isolated from THP-1 cells using RNAzol following

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Abbreviations: HSA, human serum albumin; IC, immune complex; ICAM-1, intercellular adhesion molecule-1; IL-1β, interleukin-1β; IL-8, interleukin-8; LFA-1, lymphocyte functional antigen-1; Oval, ovalbumin; PBS, phosphate-buffered saline; RT/PCR, reverse transcriptase/polymerase chain reaction; TNF-α, tumor necrosis factor-α; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4; VNR, vitronectin receptor

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the protocol described by the manufacturer (BioTecX, Houston, TX). RNA quantitation was determined by the absorbance at 260 nm. A total of 1 μg of total RNA was used as template for first strand synthesis using reverse transcriptase (RT) and oligo-d(T) and the RNA PCR kit (Perkin/Elmer, Branchburg, NJ) in a final volume of 20 μl following the procedures of the manufacturer. Following first strand synthesis, each sample was diluted 1:5 with dH_2O. 4 μl of each diluted sample was then used as template in each of the following polymerase chain reaction (PCR) amplifications. PCR primer pairs for human IL-1β, IL-8, TNF-α, and β-actin were purchased from Clontech (Palo Alto, CA). PCR reactions were performed using the RNA PCR kit and 1 μl of each primer from Clontech as recommended. The amplified samples were then subjected to agarose gel electrophoresis using a 3% agarose gel (3:1 NuSieve GTG (FMC, Rockland, ME) to ultrapure agarose (Life Technologies, Gaithersburg, MD)). Polaroid pictures were taken with Type 665 film and the negatives were scanned using a Molecular Devices Image Analyzer.

Data was quantitated by dividing each sample intensity for β-actin by the mean of intensities for β-actin ($S_{\beta act}/M_{\beta act} \times 100 = C\%$). Each mRNA specific amplified product band intensity, I, was then divided by $C\%$ ($I/C\% = I_n$) yielding the RNA specific amplified product-concentration-normalized intensity. These RNA-concentration normalized sample values (I_n) were then divided by the intensity obtained using human serum albumin (HSA) as a substrate, and subtracting 1 from all resulting numbers. $[(I_n/I_{HSA})-1]$ = percent mRNA induced above background. The final readout thus represents the message induced above adherence-induced background (i.e. HSA as a substrate).

2.2. Substrate preparation and cell culture

For preparation of substrates, proteins were isolated as follows. Fibronectin was isolated from human serum by gelatin-Sepharose, and was the kind gift of Dr. Dawn Nowlin (Tanabe Research Laboratories). The protein was diluted to 100 μg/ml in sterile dH_2O at 37°C. Fibrinogen was purchased from Sigma (St. Louis, MO), and was diluted into sterile dH_2O at 100 μg/ml. Vitronectin was purchased from Chemicon and diluted to 100 μg/ml in sterile dH_2O. Recombinant ICAM-1 was isolated from membrane extracts of S2 cells which overexpressed ICAM-1 (Cobb and Molony, unpublished results). ICAM-1 was purified by chromatography on a column of MEM111 (MediCon, Carpenteria, CA) anti-ICAM-1 eluted with 50 mM CAPS, pH 11. ICAM-1 concentration was quantitated by ELISA (R&D Systems, Cambridge, MA). ICAM-1 was used at concentrations ranging from 50-100 ng/ml. Recombinant VCAM-1 was isolated from S2 cells which overexpress human VCAM-1 [21] and used in similar concentrations as ICAM-1. Antibodies were used as pure IgG, except for anti-vitronectin receptor clone LM609, which was clarified ascites. Anti-LFA-1, anti-VLA4, anti-MAC-1, anti-ICAM-1, and anti-VCAM-1 were obtained from ImmunoTech (formerly AMAC, Inc., Cambridge, MA). Anti-β1 (AB1938) and LM609 (MAB1976) were obtained from Chemicon (Temecula, CA). All monoclonal antibodies used in this study were IgG1 isotype and were diluted from stocks of 200 μg/ml. Ovalbumin (Oval) was obtained from Sigma. Anti-ovalbumin, goat-anti-rabbit and goat-anti-mouse purified IgG were obtained from Organon Teknika/Cappel (Durham, NC). HSA was obtained from Intergen (Purchase, NY), and prepared as a 3% solution in sterile dH_2O. Antibodies were used at 2 μg/ml, except as noted for dose-response studies. Immune complex solutions were diluted from stocks which contained 100 μg/ml anti-ovalbumin IgG (Cappel) and 100 μg/ml ovalbumin. Dose-response studies used serial dilutions of a 100 μg/ml stock solution (1:1 to 1:100) of the immune complexes, consisting of the primary and secondary antibodies, which were then allowed to bind to the plates. The plates were then blocked with HSA and washed. Constant amounts of fibronectin or ovalbumin (50 μg) were then added to the appropriate wells.

All substrates were prepared by dilution into sterile dH_20 or sterile phosphate-buffered saline (PBS) (Gibco). All substrates and antibodies were sterile filtered and tested for endotoxin using ICN EndoTest (ICN, Aurora, OH) tubes prior to use. Endotoxin contaminated substrates were not used. Substrates (50 μl of each substrate solution per well) were incubated on Xenobind activated plates at room temperature for at least 2 h followed by storage overnight at 4°C. When multiple preparations were called for, plates were blocked with HSA before addition of secondary substrate or antibody. In the case of immune complex with VLA4, beta1, VNR, LFA-1, and Mac-1, the complex is composed of the primary antibody to the particular protein and the secondary antibody rather than employing isolated protein. Therefore, plates were coated with secondary antibody, blocked with HSA, washed and then incubated with primary antibody, followed by another HSA blocking step and washes. Further steps were performed according to the manufacturer (Xenopore Corp., Saddlebrook, NJ).

THP-1 cells were grown in spinner flasks or T-75 Corning flasks in RPMI (Sigma, St. Louis, MO)+glutamine (Gibco)+pen/strep (Gibco). 5% CPSR-2 (Sigma), a serum substitute, was used to reduce constitutive activation of monocytic cells. THP-1 cells were washed with RPMI (with glutamine and pen/strep) without serum or CPSR-2, and resuspended in that medium. Cells were then added to substrate or HSA coated wells and incubated at 37°C for 4 h. For measurement of IL-1β production/secretion, 2% CPSR-2 was included in the media, and the incubation time was 24 h rather than 4 h to allow synthesis of new proteins. IL-1β protein levels were measured by ELISA (Endogen, Boston, MA). The antibody used in this ELISA kit predominantly recognizes the mature form of IL-1β.

Cells were plated at 300 000–350 000 cells/well. Typically, 12 wells (1 row) of a 96-well plate were pooled for isolation of IL-1β in the supernatant and/or preparation of total RNA from the cell pellet.

3. Results

3.1. Cytokine gene induction in adherent THP-1 cells

To investigate the role of general adhesion in the induction of gene expression, non-adherent THP-1 cells were incubated in teflon troughs in RPMI media without serum. The cells were then exposed to LPS with and without serum. Total RNA from each sample was then subjected to RT/PCR to examine induced gene expression of the cytokine genes for IL-1β, IL-8, and TNF-α. The results are shown in Fig. 1. mRNA for the different cytokines was induced at varying levels depending on treatment. IL-1β, IL-8 and TNF-α gene induction was observed in the presence of LPS with and without serum.

3.2. Role of adhesion receptors of the β1 and β3 subfamilies

In order to determine the matrix specificity of IL-1β gene expression in THP-1 monocytes, we coated tissue culture flasks or pre-activated 96-well plates with a variety of substrate proteins. Cells allowed to adhere to HSA or plastic showed gene induction above the levels seen for non-adherent cells, as predicted from previous work by Rosette and Karin [22]. As can be seen in Fig. 2A, neither VCAM-1 nor vitronectin-coated plates stimulated increases of IL-1β mRNA above this background level. Adhesion of THP-1 cells to fibronectin did result in a modest induction of IL-1β mRNA. In contrast, adhesion of THP-1 cells to fibrinogen resulted in a consistent and significant induction of IL-1β mRNA.

Adherence of THP-1 cells to anti-vitronectin receptor antibodies $\alpha_v\beta_3$ (LM609) (VNR) and antibodies to the α_4 chain of VLA4 resulted in no significant upregulation of IL-1β mRNA. However, adherence to immune complexes formed by crosslinking VLA4 and VNR antibodies stimulated IL-1β mRNA production.

Similar studies were performed to determine if IL-8 mRNA was induced by adherence to matrix substrates or immune complexes (Fig. 2B). Induction of IL-8 mRNA in THP-1 cells exposed to plastic coated with HSA or any substrate, antibody, or immune complexes was minor in comparison to induction of IL-1β mRNA. Similar results were seen with TNF-α mRNA (data not shown).

3.3. Role of β2 integrins in induction of cytokine gene expression

We next examined cytokine mRNA expression as a function of β2 integrin-mediated adhesion of THP-1 cells. We allowed the cells to adhere to plastic coated with purified recombinant human ICAM-1, or to antibodies to CD11a (IOTI6), CD11b(IOM1) and ICAM-1 (84H10). As shown in Fig. 3, adherence to ICAM-1 did not result in any significant upregulation of IL-1β mRNA when compared to mRNA induced in cells plated on HSA-coated plastic, and compared to β-actin controls. In addition, no significant increase in mRNA was observed when THP-1 cells were allowed to adhere to antibodies to LFA-1, Mac-1, or ICAM-1. However, mRNA induction was observed in THP-1 cells adherent to immune complexes of anti-CD11a, anti-CD11b and anti-ICAM-1.

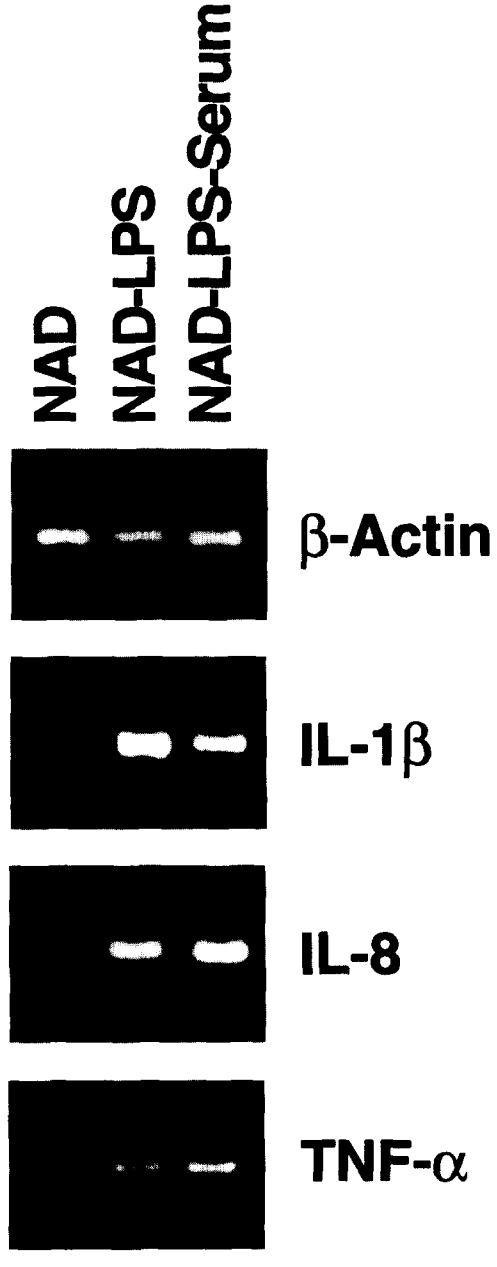

Fig. 1. RT/PCR analysis of THP-1 cells. THP-1 cells grown in Teflon coated dishes (non-adherent, NAD), treated with or without LPS. The figure is a representative experiment of four.

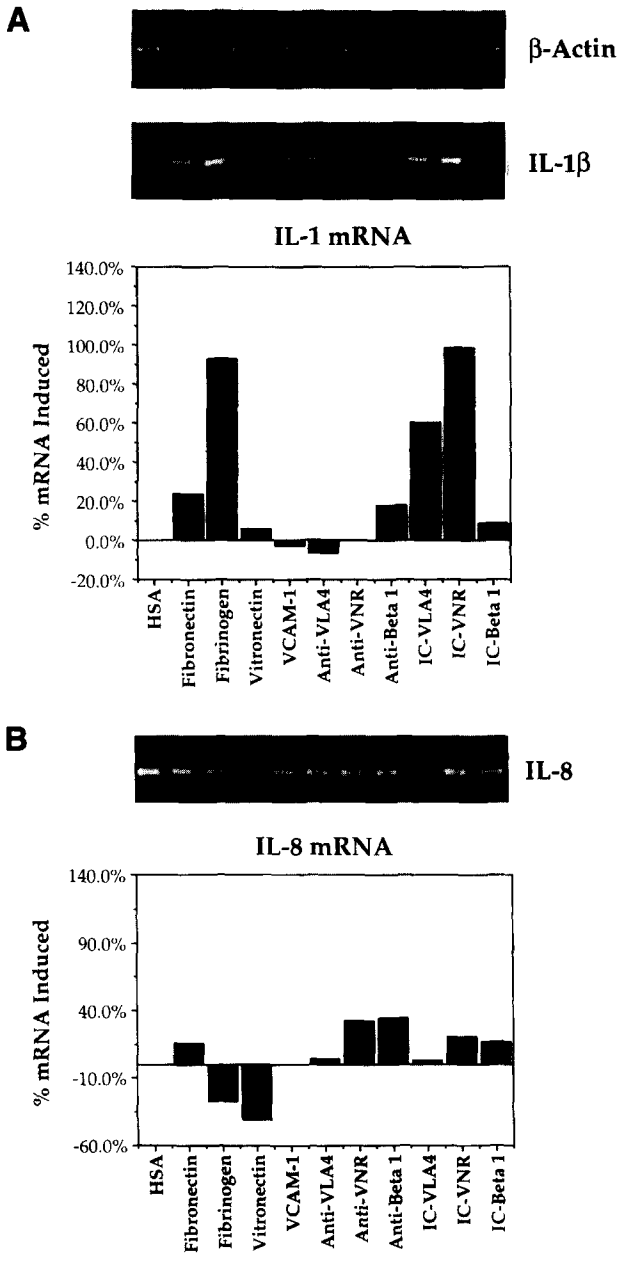

Fig. 2. Effects of β1, β3, or Fc receptor engagement on IL-1β and IL-8 gene induction. Cells were allowed to adhere to 96-well dishes coated with different substrates and RT/PCR analysis was performed after total RNA was isolated. Results are presented as percent of the normalized intensity of the RT/PCR band in the sample of the cells allowed to adhere to HSA. Each sample was repeated a minimum of four times. (A) IL-1β gene induction in THP-1 cells allowed to adhere to different substrates. (B) IL-8 gene induction in THP-1 cells allowed to adhere to different substrates.

No significant induction of IL-8 mRNA was seen as a result of cell adherence to any of the β2 integrin antibodies, substrates or immune complexes studied here (Fig. 3B). Similar results were seen with TNF-α (data not shown).

3.4. Dose response of Fc receptors to immune complex

To further investigate a possible role of the Fc receptor in upregulating gene expression, serial dilutions (1:1 to 1:100) of complexes formed with secondary antibody and primary antibody to VLA4, fibronectin, or ovalbumin were coated on 96-

well plates and subsequently blocked with HSA. A constant amount of fibronectin (50 μg) was then added to the 1:1, 1:10, and 1:100 dilutions of anti-fibronectin+anti-rabbit IgG or a constant amount of ovalbumin (50 μg) was added to the anti-ovalbumin dilutions+anti-rabbit IgG. THP-1 cells were allowed to adhere as in the other experiments and the total mRNA was isolated. As shown in Fig. 4, there was a dose-responsive increase in the amount of IL-1β mRNA observed. IL-8 and TNF-α mRNA were induced by adherence to immune complex, however, the amount of mRNA for IL-8 and TNF-α demonstrated a less pronounced dose-responsive effect as compared with IL-1β.

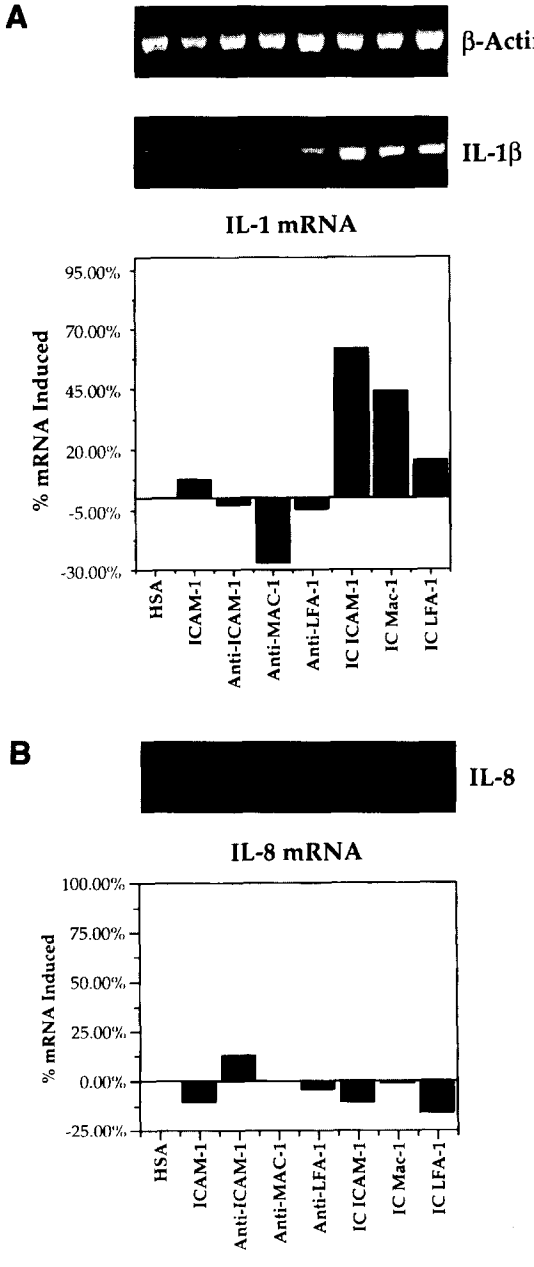

Fig. 3. Effects of β2 or Fc receptor engagement on IL-1β and IL-8 gene induction. Cells were allowed to adhere to 96-well plates coated with different substrates and RT/PCR analysis was performed after total RNA isolation. Each sample was repeated a minimum of four times. (A) IL-1β gene induction in THP-1 cells allowed to adhere to different substrates. (B) IL-8 gene induction in THP-1 cells allowed to adhere to different substrates.

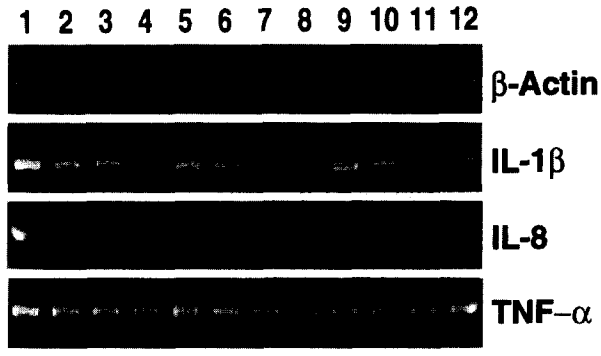

Fig. 4. Dose-response effects of immune complex on cytokine mRNA induction in THP-1 cells. THP-1 cells were allowed to adhere to the various doses of substrates as described. RT/PCR analysis was performed after total RNA was isolated. Lanes: 1, VLA4 IC 1:1; 2, VLA4 IC 1:10; 3, VLA4 IC 1:100; 4, fibronectin 50 μg/ml; 5, fibronectin+fibronectin IC 1:1; 6, fibronectin+fibronectin IC 1:10; 7, fibronectin+fibronectin IC 1:100; 8, ovalbumin 50 μg/ml; 9, ovalbumin+ovalbumin IC 1:1; 10, ovalbumin+ovalbumin IC 1:10; 11, ovalbumin+ovalbumin IC 1:100; 12, plastic alone.

3.5. IL-1β secretion is stimulated by adhesion to fibrinogen and immune complexes

To determine if production and secretion of IL-1β followed the pattern of mRNA induction by matrix, antibody or immune complex, an ELISA was performed. THP-1 cells were incubated for 24 h on matrix, antibody or immune complex. IL-1β protein present in the media at 24 h was quantitated by ELISA. The results are shown in Fig. 5. Plating THP-1 cells on HSA, plastic, and ICAM-1 did not result in IL-1β secretion. However, fibrinogen, fibronectin, as well as anti-LFA-1 IC and anti-Mac-1 IC, resulted in significant increases in IL-1β secretion after 24 h. Non-specific immune complexes, ovalbumin and anti-ovalbumin, and ovalbumin anti-ovalbumin and goat anti-rabbit immunoglobulin induced the highest levels of IL-1β.

4. Discussion

Adherence to microvascular endothelium and to extracellular matrix is an early event in the process of monocyte emigration into sites of inflammation or infection [4,23–29]. Recent studies [4,5,28] have shown that the initial adherence event can modulate the expression of inflammatory mediator genes. Previous studies have demonstrated that monocyte adherence to plastic, endothelial cells, or matrix components can result in expression of several immediate early genes [5,16,28].

Because previous studies were performed using monoclonal antibodies to specific receptors on these cells, and the corresponding F(ab) fragments recognizing these receptors did not induce gene expression, we investigated the possible role of the Fc receptors. Furthermore, previous investigations measured mRNA induction stimulated by antibody crosslinking of cells in suspension [17,18]. In the present study, we compared the relative effects of direct adhesion to immobilized substrates, which involves cytoskeletal signaling as well as receptor-specific signals [30,31], on IL-1β gene expression in THP-1 cells.

The adherence of THP-1 cells to the endothelial cell ligands ICAM-1 and VCAM-1 does not result in the induction of IL-1β mRNA accumulation. This observation implies that engagement of the β1 or β2 integrins may not be sufficient to

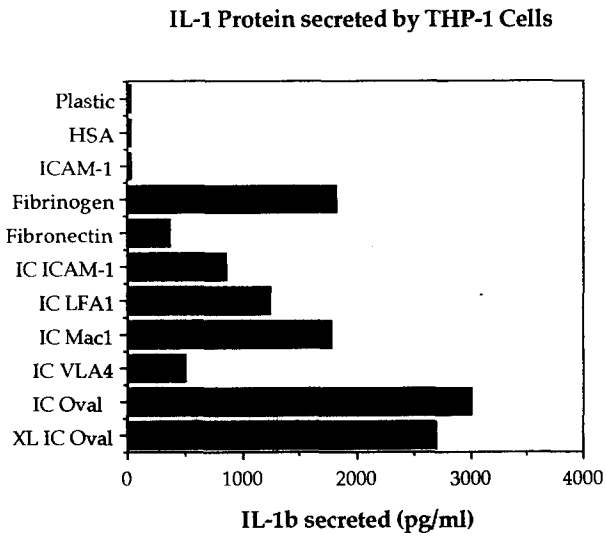

Fig. 5. IL-1β secretion in THP-1 cells exposed to various stimuli. THP-1 cells were allowed to adhere to the various substrates for 24 h. The supernatants were then isolated and assayed for IL-1β protein concentrations using an ELISA.

signal the induction of gene expression. In addition, we show that no significant induction of IL-1β mRNA accumulation relative to background was observed when THP-1 cells were allowed to adhere to monoclonal antibody coated plates. This was observed with a variety of different antibodies. The experiments presented here suggest that mere engagement of the integrin receptor is not sufficient for gene induction. Direct adhesion of THP-1 cells to matrix substrates engaging β1 and β2 integrins is also not sufficient for significant IL-1β gene induction.

Significant increases in IL-1β mRNA accumulation and protein secretion were seen when THP-1 cells were allowed to adhere to crosslinked monoclonal antibodies. This may be the result of engagement of integrin receptors in combination with recognition of immune complex by the Fc receptor. However, the highest amount of gene induction and protein secretion was observed with a non-specific immune complex. These immune complexes are recognized by the Fc receptors on a variety of cells including monocytes. These results imply that Fc receptors on THP-1 cells are primarily responsible for the increases in IL-1β gene expression observed in experiments described herein.

Our results support the conclusion that the mere engagement of individual specific integrins on the cell surface of monocytes is not sufficient for significant induction of IL-1β gene expression. The results presented also implicate the Fc receptor and the intracellular signals that result from stimulation through this receptor as primary signals that result in gene induction and the subsequent secretion of IL-1β.

Perhaps the role of adhesion in cytokine gene induction is regulatory. Secondary signals are required to induce gene expression in other systems, such as T-lymphocyte activation, where engagement of β1 or β2 integrins is a co-signal for proliferation [32–34]. It is likely that secondary signals such as endotoxin, cytokines, or immune complexes would be required to induce gene expression by monocytic cells such as THP-1. For example, evidence has supported the role of CD11b/CD18 as a regulatory integrin for Fc-mediated phagocytosis in monocytes and neutrophils [13].

Alternatively, it is possible that the requirement for secondary signals may be met by recognition of the same matrix component by two integrin receptors. This may explain our observation that THP-1 cell adherence to fibrinogen stimulated IL-1β mRNA induction and secretion. (Fibrinogen is a ligand for both β3 and β2 (Mac-1) integrins.) This possibility may also explain the results obtained by other researchers using peripheral blood monocytes, where the affinities for fibronectin binding may be similar for both the α_4 and α_5 fibronectin receptors, thus resulting in the necessary dual signals. Our results with THP-1 binding to fibronectin agrees with previous findings. In addition, the data presented here demonstrate that Fc receptor engagement significantly enhances IL-1β mRNA induction and protein secretion.

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