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Integrins as a primary signal transduction molecule regulating monocyte immediate-early gene induction

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ABSTRACT **Integrins are cell surface receptors found on monocytes that facilitate adhesion to both cellular and extracellular substrates. These integrins are thought to be involved in the selective gene induction observed after monocyte adhesion to various extracellular matrices. To investigate this hypothesis, we stimulated monocytes with monoclonal antibodies to different integrin receptors to specifically mimic the integrin receptor–ligand interactions. Engagement of the common β chain of the β_1 subfamily of integrins resulted in expression of the inflammatory mediator genes, interleukin 1β, interleukin 1 receptor antagonist, and monocyte adherence-derived inflammatory gene 6 (MAD-6), whereas engagement of the common β chain of the β_2 family did not. Furthermore, to characterize integrin-mediated gene induction, we examined the ability of antibodies to the α chain of integrin receptors to regulate gene expression. Engagement of the very late antigen 4 (VLA-4) receptor resulted in induction of all the mediator genes. Receptor crosslinking was required because individual Fab fragments were unable to stimulate gene induction whereas the divalent F(ab')$_2$ fragment and the whole IgG molecule could. Interleukin 1β secretion was dependent on the anti-integrin antibody used. Some antibodies required a second signal and, for others, direct engagement was sufficient for protein production. In conclusion, engagement of integrin receptors regulated the production of both inflammatory mediator mRNA and protein. These results suggest that integrin-dependent recognition and adherence may provide the key signals for initiation of the inflammatory response during monocyte diapedesis.**

During an inflammatory process, extravasating monocytes rapidly and transiently adhere to the endothelial cells lining the arteries and capillaries and to various extracellular and basement membrane components (1). Cell adhesion is facilitated through a family of cell surface receptors, the integrins. Integrins are $\alpha\beta$ heterodimeric glycoproteins that are responsible for cell–cell and cell–matrix interactions (2, 3). The integrin superfamily is comprised of at least eight subfamilies based on the unique structure of the various β chains (2–4). In addition to serving as a receptor responsible for mediating cell–cell or extracellular matrix (ECM) contact, integrins also link the exterior of the cell to the interior of the cell. The cytoplasmic portion of the integrins is associated with the actin cytoskeletal framework via the accessory proteins talin, vinculin, α-actinin, and possibly others (5).

Because adhesion receptors play a paramount role in the connection of a substrate/ligand to the cytoskeleton, an additional function of these transmembrane glycoproteins would be in signal transduction. Several groups have shown that the adherence of CD4$^+$ T cells to fibronectin via the very late antigen (VLA)-4 and VLA-5 integrins (6–8) or to laminin via the VLA-6 integrin (6) provides a necessary costimulatory signal that mediates CD3-dependent T-cell proliferation. Adhesion to vascular cell-adhesion molecule 1 (VCAM-1) through its receptor VLA-4 also provides a costimulatory signal for CD3-dependent activation of CD4$^+$ T cells (9, 10). Engagement of the β_2 integrin, lymphocyte function-associated antigen (LFA)-1, with its ligand, intracellular-adhesion molecule 1 (ICAM-1/CD54) (10, 11), or with monoclonal antibodies (mAbs) (12, 13) further demonstrates that these receptors act as costimulatory molecules in T-cell activation. CD2, LFA-3(CD58), and CD44 have also been implicated in mediating T-cell activation (14–16). Cell regulation in non-T cells via adhesion receptors has also been reported (17–23). Stimulation of the β_1 integrins serves as a required intermediate for interleukin 1 (IL-1)-induced alkaline phosphatase activity in osteosarcoma cells (17). Adhesion of suspension-arrested fibroblasts to fibronectin rapidly induces c-*fos* and c-*myc* expression (18), whereas adhesion of keratinocytes to fibronectin results in cell-cycle withdrawal and inhibition of terminal differentiation (19). Additional studies in fibroblasts showed that engagement of the fibronectin receptor (VLA-5) acts as an activator of the Na/H antiporter (20) and an inducer of the collagenase and stromelysin genes (21). In monocytes, LFA-3, CD44, and CD45 engagement can trigger tumor necrosis factor and IL-1 release (22) and, in carcinoma cells, β_1 integrin engagement stimulates tyrosine phosphorylation (23).

No direct evidence exists for immediate-early gene induction in monocytes via the VLA integrins, although the above mentioned work certainly suggests their potential role in signal transduction and consequent gene induction. For our work, we concentrated on the β_1 and β_2 subfamilies. The β_1 subfamily [VLA or CD49(a–f)/CD29 integrins] is primarily considered to be responsible for the adherence of cells to ECM components such as collagen, fibronectin, and laminin. This subfamily is composed of six known members, VLA-1 to VLA-6 (ref. 24). The β_2 subfamily [CD11(a–c)/CD18 integrins] is considered to be important primarily in cell–cell adherence and is composed of three members, LFA-1, Mac-1, and p150/95 (2, 3).

Adherence results in the induction of the inflammatory mediator genes IL-1β, tumor necrosis factor, and colony-stimulating factor and the protooncogene c-*fos* (25–28). Several monocyte adherence-derived (MAD) inflammatory genes are also regulated by adherence (29–31). More importantly, these early studies showed that monocyte adhesion to various ECM proteins differentially regulated the levels and types of genes induced (26, 29). In this study we wanted to

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Abbreviations: ECM, extracellular matrix; VCAM-1, vascular cell-adhesion molecule 1; LFA, lymphocyte function-associated antigen; ICAM-1, intracellular-adhesion molecule 1; IL-1, interleukin 1; MAD, monocyte adherence derived; IL-1ra, IL-1 receptor antagonist; LPS, lipopolysaccharide; mAb, monoclonal antibody; VLA, very late antigen.

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determine whether the previous results showing that adhesion regulates inflammatory mediator gene expression was due to specific integrin engagement. To examine this hypothesis, we used mAbs to the β_1 and β_2 subfamilies of integrins to stimulate monocytes nonadherently. In this report, we show that direct integrin engagement regulated steady-state mRNA levels and protein secretion.

MATERIALS AND METHODS

Isolation of Monocytes. Briefly, whole blood from random donors was diluted 1:2 in RPMI 1640 medium and centrifuged through Ficoll/Histopaque 1077 (Sigma) (32). The buffy coat cells were collected and washed with sterile isotonic saline to remove platelets. Monocytes were isolated from the rest of the buffy coat cells by centrifugation through Percoll (Pharmacia) (33), washed in sterile saline, counted, and then used at 10–20 $\times$ 10^6 cells per treatment group. In our hands, this isolation procedure does not result in monocyte activation (25). Each experiment used the monocytes isolated from one random donor.

Culture Conditions. Monocytes were cultured in endotoxin-free RPMI 1640 medium (GIBCO) at 37°C and 5% CO_2/95% air on either polystyrene tissue culture dishes (Corning) or nonadherently in polypropylene tubes (Fisher Scientific) with constant rocking for 1–4 hr, with or without mAbs depending on the experiment.

Reagents. The anti-β_1 β-subunit-specific mAb AIIB2 was a generous gift of Caroline H. Damsky (University of California, San Francisco) (21); the anti-β_1 β-subunit-specific mAbs LIA1/2, LIA1/5, TS2/16, and TS2/16 F(ab')$_2$ and the anti-VLA-4 α-subunit-specific mAbs HP1/7, HP2/1, HP2/4, HP2/4 Fab, and HP2/4 F(ab')$_2$ were a generous gift of Francisco Sanchez-Madrid and Miguel R. Campanero (Universidad Autonoma de Madrid) (34, 35). The anti-β_2 β-subunit-specific mAb 60.3 was a generous gift of John M. Harlan (University of Washington) (36) and the anti-β_2 β-subunit-specific mAbs KIM-127 and KIM-185 were a generous gift of Martyn K. Robinson (Celltech, Watertown, MA) (37). Monocytes were preincubated with the mAbs for 20 min at 4°C and then incubated for 1–4 hr at 37°C. When mAbs were added sequentially, monocytes were first pretreated at 4°C with the first mAb for 20 min, incubated for 15 min at 37°C, and then incubated with the second mAb for 1 hr at 37°C. Initial studies were carried out with saturating levels of mAbs (10 μg/ml). Subsequent studies used the mAbs at a final concentration of 1 μg/ml, as stimulation was similar when up to a 1:1000 dilution was used.

RNA Isolation and Northern Blot Analysis. Total RNA was isolated by the guanidine isothiocyanate/cesium chloride method (38). mRNA levels were determined by Northern blot analysis. Total RNA (5 μg) was electrophoresed on a 1% denaturing agarose gel and then transferred to nitrocellulose (Schleicher & Schuell) (39). Multiple blots were done from each experiment and all RNA levels were equivalent based on 18S and 28S rRNA levels (data not shown); however, all the data presented are from multiple probes of the same blot. Nitrocellulose blots were probed with ^{32}P-labeled cDNA probes made using a random-priming kit (Boehringer Mannheim). Hybridizations were incubated overnight in a 50% (vol/vol) dimethylformamide solution at 42°C. Blots were washed to a final stringency of 0.2$\times$ SSPE (1$\times$ SSPE = 0.18 M NaCl/10 mM phosphate, pH 7.4/1 mM EDTA) at 56°C and then exposed to Kodak XAR2 x-ray film (Eastman Kodak) with intensifier screens at −70°C.

Protein Quantification. Secreted IL-1β was determined by incubating isolated nonadhered monocytes for 4 hr with or without lipopolysaccharide (LPS; 10 ng/ml). Duplicate samples of supernatant from each treatment group were collected, centrifuged, and frozen at −20°C. IL-1β in culture supernatants was quantitated by a capture ELISA kit (Cistron, Pine Brook, NJ). All values represent the average of the protein secretion in pg/ml from duplicate samples. In experiments with polymyxin (10 ng/ml; Roerig Div./Pfizer), the polymyxin was added prior to monocyte incubation.

RESULTS

β_1 Integrin but Not β_2 Integrin Engagement Regulates Gene Induction. We have been interested in examining gene expression in monocytes adhering to different surfaces as a model for understanding the mechanisms involved in monocyte regulation of the inflammatory process during diapedesis (25, 26). To examine this process, we used mAbs to the common β chain of the β_1 (mAb TS2/16) and β_2 (mAb 60.3) integrins to specifically and directly mimic receptor–ligand interactions (Fig. 1). Engagement of the β_1 integrins resulted in induction of IL-1β, IL-1 receptor antagonist (IL-1ra), and MAD-6 mRNA (lane 3), whereas engagement of the β_2 integrins did not induce gene expression (lane 4). Crosslinking of the primary anti-β_2 mAb with a secondary mAb also failed to induce a signal (data not shown). Because the lack of gene induction via engagement of β_2 integrins could be a result of negative signaling or simply a lack of a signal, we examined whether preincubation of monocytes with the anti-β_2 mAb could block β_1-mediated receptor signaling. From Fig. 1, lane 6, it can be seen that prior β_2 engagement affects mRNA levels differently depending on the gene examined; no significant decrease in IL-1β expression and a modest suppression of MAD-6 expression were observed. The reverse treatment group, prior β_1 engagement (lane 5), showed no differences in IL-1β or MAD-6 but an enhancement of IL-1ra expression.

To further examine β_1-mediated signaling, a panel of anti-β_1 integrin mAbs was used. AIIB2 induced high levels of IL-1β (Fig. 2, lane 2). LIA1/2 was similarly active (lane 3),

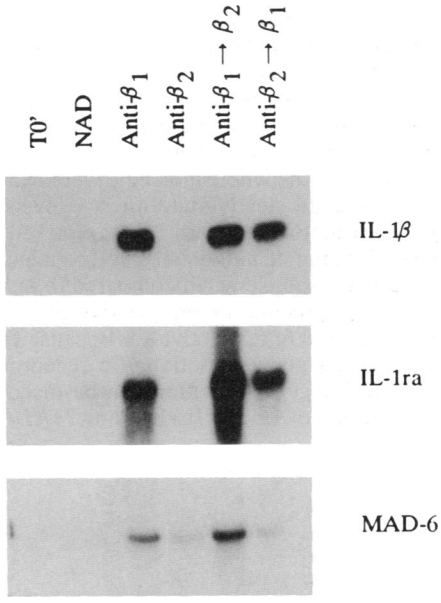

FIG. 1. β_1 but not β_2 integrin engagement with mAbs regulates immediate-early gene induction. Northern blot analysis of human monocytes treated with mAb (anti-β_1, TS2/16; anti-β_2, 60.3). Lane T0' contains monocytes harvested immediately after isolation and lane NAD contains monocytes incubated for 1 hr without mAb. mAbs were used at a final concentration of 1 μg/ml and monocytes were incubated nonadherently for 1 hr. RNA levels were equivalent based on 18S and 28S rRNA levels. All data are from a single donor and are from a representative experiment that was repeated at least three times.

but other mAbs, TS2/16 and LIA1/5, were less active (lanes 4 and 5, respectively). Stimulation was not the result of Fc binding, as the TS2/16 $F(ab')_2$ preparation was highly stimulatory (lane 6).

Because mAbs TS2/16 and 60.3 were not of the same isotype (IgG1 and IgG2a, respectively), additional β_2 mAbs were examined (Fig. 3). Other anti-β_2 mAbs did not signal mRNA expression (lanes 5 and 6). The two mAbs KIM are IgG1, thus eliminating isotype differences (37). Interestingly, KIM-127 was recently shown by Robinson *et al.* (37) to promote LFA-1 and complement receptor-3-dependent events in human B- and T-cell lines (37). Additional anti-β_2 and anti-CD11a and -CD11b mAbs also could not stimulate gene induction in monocytes (A.D.Y., unpublished data).

VLA-4 Receptor α-Chain Engagement Results in Inflammatory Gene Induction. We next investigated whether changes in mRNA levels could be elicited by direct engagement of the integrin α subunits. Monocytes were stimulated nonadherently with a panel of mAbs specific for the α subunit of VLA-4 ($\alpha_4\beta_1$). VLA-4 is a receptor for the ECM component, fibronectin, and the activated endothelial marker VCAM-1 (24, 40) and has recently been shown to be a mediator of leukocyte homotypic aggregation (35, 41, 42). Although the anti-VLA-4 α-chain-specific mAbs HP1/7, HP2/1, and HP2/4 recognize different VLA-4 epitopes (35, 42), each induced IL-1β, IL-1ra, and MAD-6 mRNA (Fig. 4). Higher levels of gene induction were observed with the two cell-aggregation-inducing mAbs, HP1/7 and HP2/4 (lanes 3 and 5, respectively), as compared to the nonaggregating mAb HP2/1 (lane 4). Monocytes adhered to plastic (lane 1) were used as a positive control (25, 26, 29). Engagement of the VLA-4 fibronectin receptor resulted in the unexpected activation of the collagen-specific gene MAD-6 (29). Isotype differences can be ruled out as the anti-VLA-4 mAbs were all the same isotype (IgG1). With some donors, a low induction of MAD-6 in the control (lane 2) was seen. This result may be due to MAD-6 being selectively induced at low levels by rapid but transient cell–cell interactions.

Divalent Receptor Crosslinking Is Necessary for mRNA Expression. Although crosslinking of the primary antibody with a secondary antibody is not necessary for the transmission of signal in monocytes, divalent crosslinking of the receptors may be necessary. Therefore, HP2/4 Fab and $F(ab')_2$ fragments and the whole molecule were examined for their ability to mediate steady-state mRNA levels (Fig. 5). Crosslinking of receptors appears to be required for gene induction since binding of individual Fab fragments (lane 4) did not stimulate a response when compared to intact whole antibody, $F(ab')_2$ fragments, or the positive plastic-adhered control (lanes 3, 5, and 6, respectively). Because the $F(ab')_2$ fragments stimulated gene induction, Fc receptor interactions as a source for gene induction can be discounted. In fact, increased levels of IL-1β, IL-1ra, and MAD-6 expression occurred with the use of the $F(ab')_2$ fragments, suggesting a potential negative signaling event may occur through the Fc receptor.

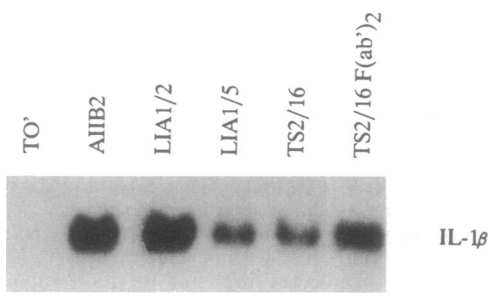

FIG. 2. A Northern blot analysis of human monocytes IL-1β induction after stimulation by a panel of mAbs demonstrating that variations exist in integrin-mediated signaling. LIA1/2, LIA1/5, and TS2/16 are anti-β_1 mAbs. Other details are as in Fig. 1.

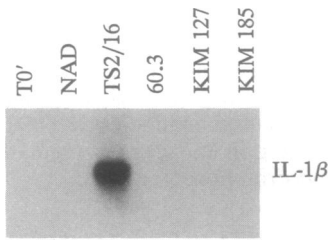

FIG. 3. Additional anti-β_2 mAbs do not induce gene expression in human monocytes. Northern blot analysis of human monocytes after stimulation by mAbs. TS2/16 is an anti-β_1 mAb and 60.3, KIM 127, and KIM 185 are anti-β_2 mAbs. Other details are as in Fig. 1 except that the experiment was repeated twice.

IL-1β Protein Secretion Is Regulated by Direct Integrin Engagement. Adherence to plastic served as a priming signal for monocytes but a second signal was needed to trigger protein production (25). In the present study, we treated nonadhered monocytes identically to those collected for mRNA expression except that monocytes were incubated for 4 hr in LPS-free medium or with low levels of LPS (10 ng/ml). From Table 1, it can be seen that two of the anti-β_1 mAbs, TS2/16 and LIA1/5, could directly stimulate IL-1β secretion without an additional secondary signal. The other mAbs, LIA1/2 and HP1/7, needed a second signal provided by LPS for monocyte protein secretion. Anti-β_2 mAb 60.3 did not stimulate protein secretion. Because the direct effect of TS2/16 and LIA1/5 on protein production could be the result of LPS contamination in the antibody fractions, polymyxin (10 μg/ml) was added to all samples. The addition of polymyxin completely inhibited IL-1β secretion in the sample groups requiring LPS as a second signal but not in the groups that did not need a second signal. This data demonstrates that integrin-mediated signaling can directly regulate protein production (primes and triggers) and that such signaling appears to be antibody-dependent since only specific antibodies elicited the observed responses.

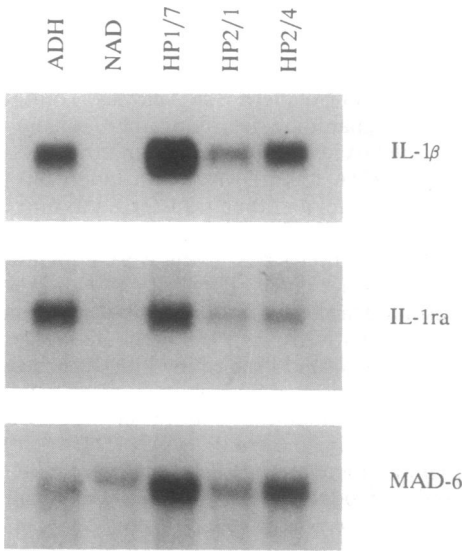

FIG. 4. VLA-4 receptor engagement regulates monocytes gene induction. Northern blot analysis of monocyte integrin engagement after stimulation with VLA-4 α chain mAbs (HP1/7, HP2/1, HP2/4). Other details are as in Fig. 1.

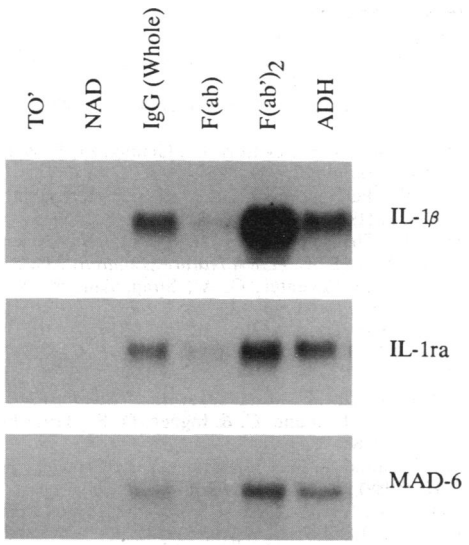

FIG. 5. Monomeric Fab fragments do not stimulate immediate-early gene induction, showing that divalent crosslinking of integrins is needed. HP2/4 whole molecule and F(ab')$_2$ and Fab fragments were used at a final concentration of 1 μg/ml. Other details are as in Fig. 1 except that the experiment was repeated at least twice.

DISCUSSION

The adherence of extravasating monocytes to endothelial cells lining the capillaries and to extracellular and basement membrane components is the first step in the path of a circulating monocyte to a site of inflammation. We have shown (25, 26, 29) that this initial adherence step modulated monocyte expression of key inflammatory mediator genes, suggesting that adherence serves as a primary regulatory stimulus. Further studies showed that adherence of monocytes to different ECM components selectively regulated gene induction (26, 29). Because integrins selectively regulate adhesion to different substrates and have been implicated by others to be potential signal transduction molecules in other cell types (6–13, 17–21, 23), we investigated their potential role in specific monocyte adherence-mediated signaling and consequent gene induction.

By using direct receptor engagement as a model, we showed that engagement of β_1 but not β_2 integrins induced mRNA expression. Differences in gene expression were not due to mAb isotype differences but rather appear to be due to the lack of signaling (leading to mRNA expression). Furthermore, preliminary studies with soluble ICAM-1 also showed no gene induction (A.D.Y., unpublished data). This lack of a signal generated by the anti-β_2 mAbs does not appear to be due to differences in the rate mAbs are internalized, as cells labeled with anti-β_1 and anti-β_2 mAbs and then incubated showed similar fluorescent surface patterns.

Studies with T cells showing that the β_2 integrin LFA-1 acts as a costimulatory molecule were done with immobilized anti-LFA-1 (12) or ICAM-1 (10, 11) or crosslinked anti-LFA-1 (13), suggesting β_2 receptor crosslinking and perhaps events regulated by adherence itself or some other costimulatory signal are necessary for a response. Furthermore, these studies showed that β_2 engagement only provided a costimulatory signal and that a second signal was needed. Diamond *et al.* (43) showed that LFA-1 and Mac-1 bind to different domains of ICAM-1 and suggest that each could provide a unique signal (43). This latter possibility seems likely, because while investigating whether β_2 integrins may send a negative signal rather than no signal, we showed that preincubation of cells with anti-β_2 prior to anti-β_1 modestly down-regulated MAD-6 expression but not IL-1β expression.

Table 1. IL-1β secretion by mAb-stimulated monocytes

	IL-1β secreted, pg/ml					
mAb	1	2	3A	3B	3C	3D
NAD	9.2	L	L	L	L	99.6
TS2/16	101.6	155.2	102.4	31.7	29.8	351.2
LIA1/2	18.8	L	L	L	L	886.1
LIA1/5	288.7	188.8	184.8	50.8	84.7	711.0
HP1/7	25.2	L	L	L	8.3	320.7
60.3	14.4	L	L	L	L	94.0

TS2/16, LIA1/2, LIA1/5 are anti-β_1 mAbs; HP1/7 is an anti-VLA-4 mAb; and 60.3 is an anti-β_2 mAb. In experiments 1, 2, and 3A, monocytes were stimulated without LPS. Experiments 1, 2, and 3A are identical experiments with different donors. Values represent averages from duplicate samples measured by ELISA. In experiment 3B, monocytes were treated as experiment 3A except that polymixin (10 ng/ml) was added. Experiment 3C is the same as 3A except that polymixin (10 ng/ml) plus LPS (10 ng/ml) were added. Experiment 3D is the same as 3A except that LPS (10 ng/ml) was added. L indicates a value below the limit of detection.

The differential effects of prior β_2 compared with prior β_1 engagement could be the result of differences in aggregation; however, additional studies showed that there were no differences in the number of monocytes aggregated after incubation with either anti-β_1 or anti-β_2 mAbs (A.D.Y., unpublished data). These data suggest that β_2 engagement in monocytes may specifically transmit a signal, but the signal transmitted does not directly induce mRNA and may need a costimulatory signal to mediate a full response.

To further clarify the mechanisms involved in receptor signaling, mAbs to the α chain of the VLA-4 receptor were used to trigger mRNA expression. The VLA-4 receptor is one of the three β_1 fibronectin receptors (24). It recognizes the CS-1 segment of fibronectin, independent of the RGD amino acid sequence, the recognition site for VLA-5 (44). VLA-4 is atypical because it also recognizes the endothelial cell marker VCAM-1 and may be directly responsible for leukocyte homotypic aggregation (24, 35, 40–42). Gene expression was induced by engagement of the VLA-4 receptor. The importance of the VLA-4 receptor and signaling through it comes from studies of patients with β_2 adhesion deficiencies (leukocyte adhesion deficiency) in which neutrophils fail to move to sites of inflammation, whereas monocytes and lymphocytes move normally (3, 45). VLA-4 is present on monocytes and lymphocytes but not neutrophils and may be the key adherence receptor for monocyte and lymphocyte trafficking (46).

In monocytes, signaling through the VLA integrins requires only divalent crosslinking and not the addition of secondary antibody to crosslink. This has also been observed in keratinocyte differentiation (19) and in studies examining collagenase and stromelysin expression in fibroblasts, after stimulation with anti-fibronectin receptor antibodies (21). This lack of necessity for secondary crosslinking of integrins is in contrast to T-cell (6–16) and other systems (23), where immobilization or secondary crosslinking and/or costimulation is necessary for signaling.

MAD-6 is a collagen- but not fibronectin-selective gene described by Sporn *et al.* (29) and is equivalent to the A-20 zinc finger apparent transcription factor (which inhibits programmed cell death) (50). Engagement of the VLA-4 receptor unexpectedly induced MAD-6 mRNA. One possible explanation for this may be that integrin engagement with the biological ligand fibronectin results in a unique set of signals that are different from those generated by the anti-VLA-4 antibodies. This possible explanation is supported by a study (21) that observed that different signals were generated depending on whether the natural fibronectin ligand, fibronectin fragments, or antibodies to the fibronectin receptor

were used. Alternatively, because VLA-4 binds VCAM-1, we could be mimicking the VCAM-1–VLA-4 interactions with the anti-VLA-4 mAbs. Our previous observation that monocytes adhered to an endothelial cell line express MAD-6 (29) raises the possibility that VCAM-1–VLA-4 interactions induce the putative transcription factor MAD-6.

Finally in the present study, we addressed protein secretion stimulated by integrin engagement. Adherence has been shown to prime monocytes and a second signal was needed to induce protein production (25). In this work, we demonstrated that two anti-β_1 mAbs directly stimulate IL-1β secretion without an additional secondary signal. Adherence to plastic may result in different signaling pathways than those seen after β_1 integrin engagement [preliminary phosphorylation studies support that conclusion (A.D.Y., unpublished data)]. The differences between the responses generated after treatment with various antibodies may be due to differences in the affinity of binding of the individual antibodies. Alternatively, it could be due to differences in signaling based on the unique epitopes recognized by the various mAbs. This suggestion that different epitopes regulate different responses is supported by the work of Pulido *et al.* (42) who described different functional epitopes on at least the VLA-4 integrin. The anti-β_2 mAb did not stimulate protein secretion, supporting the conclusion that β_2 engagement does not provide the necessary signals for mRNA expression or protein secretion. These data also provide additional evidence that LPS was not contaminating our monocytes or mAb stocks, because many of the mAbs without exogenously added LPS could not stimulate protein secretion but did elicit IL-1β transcripts.

Thus these results clearly demonstrate that direct integrin engagement in monocytes regulates both steady-state mRNA and protein levels. From our present data, one could envision that, as an extravasating monocyte adheres to the cells lining the capillaries and then to the ECM through the β_1 integrins, inflammatory mediator genes and products would be induced, resulting in a local and perhaps systemic response. The importance of fully understanding this pathway is clear, especially in cases of chronic inflammatory diseases where increased amounts of fibronectin or collagen can be found (47, 48). Increased amounts of matrix components could cause hyper or aberrant signaling through adhesion receptors, resulting in an unregulated or an uncontrolled inflammatory response. These results emphasize the central role of the common β_1 chain in regulating inflammatory responses (49).

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1. Osborn, L. (1990) *Cell* **62**, 3–6.
2. Albelda, S. M. & Buck, C. A. (1990) *FASEB J.* **4**, 2868–2880.
3. Springer, T. A. (1990) *Nature (London)* **346**, 425–434.
4. Moyle, M., Napier, M. A. & Mclean, J. W. (1991) *J. Biol. Chem.* **266**, 19650–19658.
5. Burridge, K., Fath, K., Kelly, T., Nuckolls, G. & Turner, C. (1988) *Annu. Rev. Cell Biol.* **4**, 487–525.
6. Shimizu, Y., Van Seventer, G. A., Horgan, K. J. & Shaw, S. (1990) *J. Immunol.* **145**, 59–67.
7. Davis, L. S., Oppenheimer-Marks, N., Bednarczyk, J. L., McIntyre, B. W. & Lipsky, P. E. (1990) *J. Immunol.* **145**, 785–793.
8. Nojima, Y., Humphries, M. J., Mould, A. P., Komoriya, A., Yamada, K. M., Schlossman, S. F. & Morimoto, C. (1990) *J. Exp. Med.* **172**, 1185–1192.
9. Damle, N. K. & Aruffo, A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6403–6407.
10. Van Seventer, G. A., Newman, W., Shimizu, Y., Nutman, T. B., Tanaka, Y., Horgan, K. J., Gopal, T. V., Ennis, E., O'Sullivan, D., Grey, H. & Shaw, S. (1991) *J. Exp. Med.* **174**, 901–913.
11. Van Seventer, G. A., Shimizu, Y., Horgan, K. J. & Shaw, S. (1990) *J. Immunol.* **144**, 4579–4586.
12. Wacholtz, M. C., Patel, S. S. & Lipsky, P. E. (1989) *J. Exp. Med.* **170**, 431–448.
13. Pardi, R., Bender, J. R., Dettori, C., Giannazza, E. & Engleman, E. G. (1989) *J. Immunol.* **143**, 3157–3166.
14. Bierer, B. E., Peterson, A., Gorga, J. C., Herrmann, S. H. & Burakoff, S. J. (1988) *J. Exp. Med.* **168**, 1145–1156.
15. Moingeon, P., Chang, H.-C., Wallner, B. P., Stebbins, C., Frey, A. Z. & Reinherz, E. L. (1989) *Nature (London)* **339**, 312–314.
16. Shimizu, Y., Van Seventer, G. A., Siraganian, R., Wahl, L. & Shaw, S. (1989) *J. Immunol.* **143**, 2457–2463.
17. Dedhar, S. (1989) *Exp. Cell Res.* **183**, 207–214.
18. Dike, L. E. & Farmer, S. R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6792–6796.
19. Adams, J. C. & Watt, F. M. (1989) *Nature (London)* **340**, 307–309.
20. Schwartz, M. A., Lechene, C. & Ingber, D. E. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 7849–7853.
21. Werb, Z., Tremble, P. M., Behrendtsen, O., Crowley, E. & Damsky, C. H. (1989) *J. Cell Biol.* **109**, 877–889.
22. Webb, D. S. A., Shimizu, Y., Van Seventer, G. A., Shaw, S. & Gerrard, T. L. (1990) *Science* **249**, 1295–1297.
23. Kornberg, L. J., Earp, H. S., Turner, C. E., Prockop, C. & Juliano, R. L. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 8392–8396.
24. Hemler, M. E. (1990) *Annu. Rev. Immunol.* **8**, 365–400.
25. Haskill, S., Johnson, C., Eierman, D., Becker, S. & Warren, K. (1988) *J. Immunol.* **140**, 1690–1694.
26. Eierman, D. F., Johnson, C. E. & Haskill, J. S. (1989) *J. Immunol.* **142**, 1970–1976.
27. Fuhlbrigge, R. C., Chaplin, D. D., Kiely, J.-M. & Unanue, E. R. (1987) *J. Immunol.* **138**, 3799–3802.
28. Thorens, B., Mermod, J.-J. & Vassalli, P. (1987) *Cell* **48**, 671–679.
29. Sporn, S. A., Eierman, D. F., Johnson, C. E., Morris, J., Martin, G., Ladner, M. & Haskill, S. (1990) *J. Immunol.* **144**, 4434–4441.
30. Haskill, S., Martin, G., Van Le, L., Morris, J., Peace, A., Bigler, C. F., Jaffe, G. J., Hammerberg, C., Sporn, S. A., Fong, S., Arend, W. P. & Ralph, P. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3681–3685.
31. Haskill, S., Beg, A. A., Tompkins, S. M., Morris, J. S., Yurochko, A. D., Sampson-Johannes, A., Mondal, K., Ralph, P. & Baldwin, A. S. (1991) *Cell* **65**, 1281–1289.
32. Boyum, A. (1968) *Scand. J. Clin. Lab. Invest.* **21** Suppl. 97, 77–89.
33. Ulmer, A. J. & Flad, H.-D. (1979) *J. Immunol. Methods* **30**, 1–10.
34. Hemler, M. E., Sanchez-Madrid, F., Flotte, T. J., Krensky, N. M., Burakoff, S. J., Bhan, A. K., Springer, T. A. & Strominger, J. L. (1984) *J. Immunol.* **132**, 3011–3018.
35. Campanero, M. R., Pulido, R., Ursa, M. A., Rodriguez-Moya, M., de Landazuri, M. O. & Sanchez-Madrid, F. (1990) *J. Cell Biol.* **110**, 2157–2165.
36. Wallis, W. J., Hickstein, D. D., Schwartz, B. R., June, C. H., Ochs, H. D., Beatty, P. G., Klebanoff, S. J. & Harlan, J. M. (1986) *Blood* **67**, 1007–1013.
37. Robinson, M. K., Andrew, D., Rosen, H., Brown, D., Ortlepp, S., Stephens, P. & Butcher, E. C. (1992) *J. Immunol.* **148**, 1080–1085.
38. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18**, 5294–5299.
39. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5201–5205.
40. Elices, M. J., Osborn, L., Takada, Y., Crouse, C., Luhowsky, S., Hemler, M. E. & Lobb, R. R. (1990) *Cell* **60**, 577–584.
41. Bednarczyk, J. L. & McIntyre, B. W. (1990) *J. Immunol.* **144**, 777–784.
42. Pulido, R., Elices, M. J., Campanero, M. R., Osborn, L., Schiffer, S., Garcia-Pardo, A., Lobb, R., Hemler, M. E. & Sanchez-Madrid, F. (1991) *J. Biol. Chem.* **266**, 10241–10245.
43. Diamond, M. S., Staunton, D. E., Marlin, S. D. & Springer, T. A. (1991) *Cell* **65**, 961–971.
44. Wayner, E. A., Garcia-Pardo, A., Humphries, M. J., McDonald, J. A. & Carter, W. G. (1989) *J. Cell Biol.* **109**, 1321–1330.
45. Anderson, D. C. & Springer, T. A. (1987) *Annu. Rev. Med.* **38**, 175–194.
46. Holtzmann, B., McIntyre, B. W. & Weissman, I. L. (1989) *Cell* **56**, 37–46.
47. Carsons, S., Mosesson, M. W. & Diamond, H. S. (1981) *Arthritis Rheum.* **24**, 1261–1267.
48. Stecher, V. J., Kaplan, J. E., Connolly, K., Mielens, Z. & Saelens, J. K. (1986) *Arthritis Rheum.* **29**, 394–399.
49. Arroyo, A. G., Sánchez-Mateos, P., Campanero, M. R., Martín-Padura, I., Dejana, E. & Sánchez-Madrid, F. (1992) *J. Cell Biol.* **117**, 659–670.
50. Opipari, W. W. Jr., Laherty, C. D., Krikos, A. & Dixit, V. M. (1992) *FASEB* **6**, A1780.