Cell adhesion regulates gene expression at translational checkpoints in human myeloid leukocytes

Tracey S. Mahoney*, Andrew S. Weyrich*†, Dan A. Dixon*‡§, Thomas McIntyre*†, Stephen M. Prescott†‡§, and Guy A. Zimmerman*†¶

*The Eccles Program in Human Molecular Biology and Genetics, ‡The Huntsman Cancer Institute, and Departments of †Internal Medicine and Experimental Pathology and §Oncological Sciences, University of Utah School of Medicine, Salt Lake City, UT 84112

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Engagement of adhesion molecules on monocytes and other myeloid leukocytes, which are effector cells of the innate immune system, not only tethers the leukocytes in place but also transmits outside-in signals that induce functional changes and alter gene expression. We found that a subset of mRNAs that are induced or amplified by adhesion of human monocytes to P-selectin via its surface ligand, P-selectin glycoprotein 1, have characteristics that suggest specialized translational control. One of these codes for urokinase plasminogen activator receptor (UPAR), a critical surface protease receptor and regulator of cell adhesion and migration. Although UPAR transcripts are induced by adhesion, rapid synthesis of the protein uses constitutive mRNA without a requirement for new transcription and is regulated by mammalian target of rapamycin, demonstrating new biologic roles for the signal-dependent translation pathway controlled by this intracellular kinase. The synthesis of UPAR in monocytic cells is also regulated by eukaryotic translation initiation factor 4E, a second key translational checkpoint, and phosphorylation of eukaryotic translation initiation factor 4E is induced by adhesion of monocytes to P-selectin. Translationally controlled display of UPAR by monocytes confers recognition of the matrix protein, vitronectin. Adhesion-dependent signaling from the plasma membrane to translational checkpoints represents a previously unrecognized mechanism for regulating surface phenotype that may be particularly important for myeloid leukocytes and other cells that are specialized for rapid inflammatory and vascular responses.

Altered expression of gene products in response to signals from the environment is required for physiologic cellular functions and also occurs in disease. Signal-dependent transcriptional regulation has been intensely studied (1). In contrast, posttranscriptional checkpoints that are regulated by outside-in signals are not well characterized, although current evidence indicates that they are involved in pathologic inflammation and tumorigenesis (2). Signal-induced translation of mRNAs that code for highly regulated proteins with potent or essential biologic actions is an important posttranscriptional mechanism in development and cell replication (3–5). Pathways from surface receptors to intracellular translational checkpoints have been characterized in model systems and a few primary human cells (4, 6). One specialized pathway involves mammalian target of rapamycin (mTOR; also termed FRAP and RAFT-1), a kinase that is required for cell cycle progression and cell growth and that is activated by an intracellular signaling cascade linked to surface receptors for soluble growth factors and mitogens (3, 7). Eukaryotic translation initiation factor 4E (eIF4E), a second major translational checkpoint, is controlled by regulatory proteins termed eIF4E-binding proteins that are phosphorylated by mTOR; eIF4E activity is also influenced by phosphorylation on a serine residue in response to parallel signals that are independent of mTOR (6, 8).

Myeloid leukocytes are specialized effector cells of the innate immune system. The monocyte, a particular myeloid leukocyte, synthesizes new gene products in defensive inflammation, wound repair, immune surveillance, and diverse pathologic syndromes including sepsis and atherosclerosis (9–12). Human monocytes are also general models for study of mechanisms of gene expression in response to signals from the environment (9–11). Engagement of adhesion (tethering) molecules on the surfaces of monocytes and other leukocytes delivers outside-in signals, in addition to targeting and localizing the cells to specific sites. This provides a mechanism for spatially regulated signaling (13). P-selectin glycoprotein 1 (PSGL-1), a plasma membrane tethering molecule, mediates adhesion of monocytes and other myeloid leukocytes to activated platelets and endothelial cells that display P-selectin (14). PSGL-1 also delivers outside-in signals to mitogen-activated protein kinase pathways and induces transcriptionally regulated gene expression when it is engaged on monocytes and neutrophils (10, 11, 14, 15). Here, we show that engagement of PSGL-1 not only delivers transcriptional signals but also induces rapid synthesis of key gene products by signaling to mTOR and eIF4E, and we demonstrate that mTOR acutely regulates cell surface phenotype. Signal-dependent translational control, which has not been previously identified in myeloid leukocytes, may be particularly important to monocytes and other specialized blood cells (16, 17) that must rapidly and precisely respond in inflammation, hemostasis, and repair.

Materials and Methods

Reagents and Antibodies. Solutions were from BioWhittaker. Human serum albumin (25%) was from Baxter Health Care (Mundelein, IL). Rapamycin was from Alexis (San Diego). Purified P-selectin and mAbs PL-1 and PL-2 (IgG and Fab forms) against PSGL-1 (15) were generous gifts from Rod McEver (Univ. of Oklahoma, Oklahoma City). Antibodies against urokinase plasminogen activator receptor (UPAR) (mAb 3937, mAb VIMS, polyclonal Ab 399R) were from American Diagnostica (Greenwich, CT) or PharMingen, an mAb against integrin $\alpha_{II}\beta_3$ (7E3) was from Centocor, an mAb against $\alpha v\beta 3$ (mAb 1976) was from Chemicon, an mAb against eIF4E and a control cell lysate (SKN) were from Transduction Laboratories (Lexington, KY), and an mAb against actin was from Calbiochem. All other reagents and proteins were from Sigma.

Cells. Human monocytes were purified (90–95%) by countercurrent elutriation to avoid activation events triggered by adhesion to plastic or other substrates as described (10, 11). Collection of

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Abbreviations: mTOR, mammalian target of rapamycin; eIF4E, eukaryotic translation initiation factor 4E; PSGL-1, P-selectin glycoprotein ligand 1; LPS, lipopolysaccharide; 5′ UTR, 5′ untranslated region; UPAR, urokinase plasminogen activator receptor.

¶To whom reprint requests should be addressed. E-mail: guy.zimmerman@hmbg.utah.edu.

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blood samples from human volunteers was approved by the University of Utah Institutional Review Board. U937 myelomonocytic cells were obtained from the American Type Tissue Collection.

Interrogation of Arrayed Libraries and Transcript Analysis. Monocytes isolated by elutriation were immediately lysed, allowed to adhere to wells precoated with purified P-selectin (2.5 μg/μl) for 1 h at 37°C as described (10, 11), or incubated as described in the text and figures before isolation of mRNA by a modification of described methods (16). Poly(A) RNA was purified (Qiagen, Chatsworth, CA), and radiolabeled cDNA was synthesized and used to probe an arrayed library of human cDNA fragments (CLONTECH) according to the manufacturer's directions. Hybridization patterns of mRNA isolated from monocytes adherent to P-selectin were compared with those from freshly isolated monocytes not subjected to adhesion (as described in *Results*) and, in some cases, to stimulated monocytes and monocyte-derived macrophages (T.S.M., S.-K. An, G.A.Z., unpublished observations). Reverse transcriptase–PCR and RNase protection were done as described (16). Primer sets used for UPAR analysis by reverse transcriptase–PCR were specific to the coding region and were compared with primers for glyceraldehyde-3-phosphate dehydrogenase in optimized assays. The 5′ untranslated regions (5′ UTR) of transcripts were analyzed by using the MACDNASIS program (Hitachi, Tokyo) to identify features indicating translational regulation (see text).

Western Blotting. Elutriated monocytes were resuspended in serum-free M199 containing polymixin B sulfate [unless stimulated with lipopolysaccharide (LPS)] and allowed to adhere to immobilized P-selectin, collagen I (50 μg/ml), collagen IV (50 μg/ml), laminin (25 μg/ml), fibronectin (30 μg/ml), or albumin (1 mg/ml) by using wells coated with these proteins as described (10, 16). Where indicated, LPS (1 μg/ml), phorbol 12-myristate 13-acetate (10 nM), rapamycin (100 nM), wortmannin (100 nM), or actinomycin D (10 μg/ml) was added to the medium. Monocytes were allowed to adhere for 4 h at 37°C unless otherwise indicated before harvesting in 1 × Laemmli sample buffer. Proteins in the leukocyte lysates were resolved by SDS/PAGE and immunoblotted by using antibodies against UPAR (antibodies 3937 or 399R), actin, or eIF4E as indicated, followed by incubation with the appropriate goat anti-mouse or goat anti-rabbit peroxidase-conjugated secondary antibodies (BioSource International, Camarillo, CA). The blots were developed by using enhanced chemiluminescence (Amersham Pharmacia) (17, 18).

Construction of eIF4E-Overexpressing Leukocytes. The coding region for human eIF4E (GenBank accession no. M15353) was amplified by using PCR (primer set, 5′-CAGATCGATCTAAGATGGCG-3′ and 5′-GTCTTCTTAAACAACAAACC-3′) and subcloned (TA cloning kit, Invitrogen) into the pcDNA-3 eukaryotic expression vector (Invitrogen). The integrity and orientation of the eIF4E cDNA was confirmed by PCR, sequencing, and *in vitro* translation. U937 cells were transfected by using a Bio-Rad Gene Pulser using 15 μg of linearized construct. Cells were cultured in medium containing G418 (1.5 mg/ml) and analyzed after all untransfected U937 cells, cultured in parallel, were killed by this regimen.

For affinity purification of eIF4E, stably transfected cells were harvested by centrifugation and lysed in buffer (50 mM β-glycerophosphate/50 mM sodium fluoride/1.5 mM EDTA) containing 10 μg/ml aprotinin, pepstatin, and leupeptin, 1 mM DTT, and 1 mM sodium orthovanadate. Fractions of the lysate or the soluble portion were normalized for protein and incubated with 7-methyl GTP-Sepharose 4B beads (Amersham Pharmacia) for 30 min at ambient temperature. After affinity purification, the beads were then washed twice and resuspended in Laemmli buffer before SDS/PAGE.

Vertical Slab Isoelectric Focusing. Vertical slab isoelectric focusing was accomplished by using a modification of a previously described method (19). Immunoblotting for eIF4E was performed as described above for UPAR.

Adhesion Assays. A previously reported assay was used to measure UPAR-mediated adhesion of monocytes to vitronectin (20) under conditions outlined in the text and Fig. 4. In each experiment, values for adhesion were normalized to a maximal number of adherent cells induced by engagement of PSGL-1 to trigger UPAR synthesis and subsequent stimulation with LPS (see text).

Results and Discussion

Adhesion of Monocytes to P-Selectin Induces or Amplifies Multiple mRNAs, Including Transcripts That Are Predicted to Be Under Translational Control by mTOR. To identify genes in human monocytes that are induced in an adhesion-dependent fashion and are also regulated at the level of translation, we first interrogated an arrayed cDNA library by using probes generated from mRNA extracted from monocytes isolated by countercurrent elutriation and allowed to adhere to purified P-selectin under conditions in which PSGL-1 is specifically engaged (10, 11). The pattern of transcripts in monocytes adherent to P-selectin was then compared with that in freshly isolated monocytes, which served as the comparative condition for transcripts present in the basal state in the absence of adhesion. Multiple mRNAs (≈5% of the genes in the arrayed library that we used) were induced or amplified (2-fold or greater over base line) (Table 1). Subsequent experiments validated the observation that specific transcripts are differentially expressed when monocytes adhere to P-selectin (see below; T.S.M. and D.A.D., unpublished data).

We then examined the sequences of the 5′ UTRs of the transcripts in our array analysis for features that predict translational regulation by the mTOR pathway. These include consecutive pyrimidine residues (polypyrimidine-rich tracts) and extensive secondary structure of the 5′ UTR with calculated stabilities greater than −50 Kcal/mol (3, 5, 7, 21). A subset of the transcripts amplified in monocytes adherent to P-selectin have these features (Table 1). One of these is the mRNA for UPAR, which we chose for further analysis because it has both polypyrimidine-rich tracts and extensive secondary structure in its 5′ UTR (22). Furthermore, its protein product is extensively regulated and mediates coordinated cell adhesion and migration by binding and localizing the action of urokinase plasminogen activator (UPA), a protease involved in inflammation, angiogenesis, and neoplasia, by recognizing vitronectin, and by other mechanisms (23–25). Thus, the UPAR gene product has characteristics that make it a likely candidate for specialized translational control (26). For comparison with UPAR, we chose IL-8, a proinflammatory chemokine that is synthesized when PSGL-1 is engaged on myeloid leukocytes (10, 11, 15) but does not have sequence features in its 5′ UTR that indicate regulation by mTOR (Table 1).

Rapid Synthesis of UPAR Protein Is Induced by Adhesion of Monocytes to P-Selectin. UPAR transcripts were present in freshly isolated monocytes and were increased in monocytes adherent to P-selectin when analyzed by reverse transcriptase–PCR and RNase protection assays, consistent with the array analysis outlined above (Fig. 1*a* and data not shown). Transcripts for UPAR were also increased when monocytes were incubated in suspension in parallel as a control for *ex vivo* manipulation, but the levels were

Table 1. Induced transcripts in monocytes adherent to P-selectin identified by arrayed cDNA library analysis

Gene	SSE kcal/mol
*UPAR	−215
*CYP1B1	−185
Thrombomodulin	−99
Interleukin-4 receptor alpha chain	−71
Transcription factor IL-4 STAT	−70
Cytoplasmic antiproteinase 3	−61
*Tyrosine protein kinase HCK	−60
*CD157	−53
Cyclin-dependent kinase inhibitor 1a	−46
*Insulin-like growth factor 1A	−35
CXCCR4	−33
*Protein kinase CLK (CLK1)	−30
MCP-1	−27
MIP-1 alpha	−27
Transcription factor p65	−25
*MIP-1 beta	−21
Bcl 2A1	−21
*IL-1 receptor type II	−20
TNF-inducible protein	−19
Interleukin-8	−18
HM74	−17
G_i3 guanine nucleotide regulatory protein	−15
*Interleukin-1 beta	−14
*MIP-2 alpha	−12
Protein kinase C delta	?

Transcripts that were induced or increased (at least 2-fold over base-line levels) when mRNA isolated from monocytes adherent to P-selectin was analyzed using an arrayed cDNA library are shown. The factors are ranked according to the likelihood that the transcript is subject to translational control based on predicted secondary structure energy (SSE) of the 5′ UTR. Asterisks indicate one to three tracts consisting of 6–11 consecutive pyrimidine residues in the 5′ UTR. See text for details.

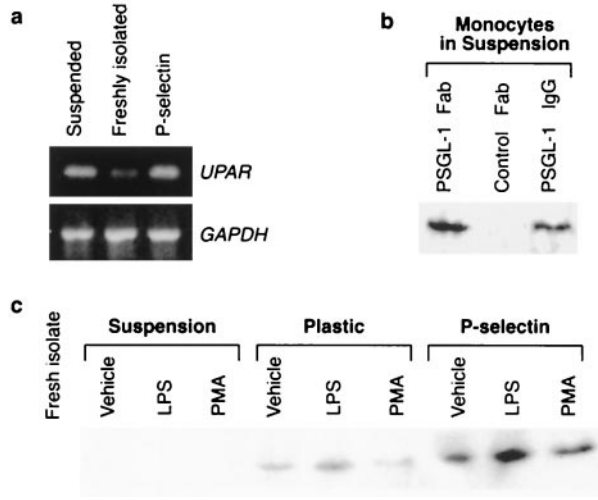

Fig. 1. UPAR transcripts are constitutively present in monocytes, and the protein is synthesized when PSGL-1 is engaged. (*a*) Expression of RNA for UPAR and glyceraldehyde-3-phosphate dehydrogenase was assayed by reverse transcriptase–PCR in freshly isolated monocytes or monocytes incubated while adherent to P-selectin or incubated in suspension for 1 h. The figure is representative of four additional experiments. (*b*) Monocytes were incubated in suspension with an Fab fragment of the anti-PSGL-1 mAb PL-1, with a control Fab that binds to myeloid leukocytes (46), or with full-length PL-1 for 4 h at 37°C. They were then lysed, and equivalent amounts of protein were immunoblotted with mAb 399R against UPAR. In a second experiment, a different mAb against PSGL-1 that triggers outside-in signals, PL-2 (15), also induced UPAR synthesis. (*c*) Monocytes were lysed immediately after isolation, after incubation in suspension for 4 h, or after adherence to plastic surfaces or to purified immobilized P-selectin for 4 h. Some replicates were treated with LPS or phorbol 12-myristate 13-acetate during the incubation. Proteins from equivalent amounts of cell lysate were immunoblotted for UPAR. Induction of UPAR protein by adhesion of monocytes to P-selectin was seen in multiple additional experiments. LPS, as a second signal, further enhanced UPAR accumulation.

less than in cells adherent to P-selectin (Fig. 1*a* and data not shown). In contrast to mRNA, UPAR protein was not detectable by Western analysis in freshly isolated monocytes or monocytes incubated in suspension (Figs. 1 *b* and *c*, 2, and 3). This differential expression of the UPAR transcript and protein suggests translational regulation (4, 5, 26) (see below).

We further examined monocytes by using immunocytochemical analysis and confocal microscopy and detected no UPAR protein in freshly isolated cells, in monocytes incubated in suspension, or in mixed leukocyte suspensions before elutriation; in contrast, there was clear staining of monocytes adherent to immobilized P-selectin (not shown). We also examined heterotypic aggregates of monocytes adherent to thrombin-stimulated platelets, a condition in which P-selectin on the platelet surface engages PSGL-1 on the monocyte and delivers outside-in signals (10, 11), and found that UPAR is present in monocytes under these conditions (not shown). UPAR was not detected in monocytes treated with thrombin or when incubated with control platelets. These experiments suggested that synthesis of the UPAR gene product is triggered when PSGL-1 on monocytes is ligated by P-selectin. Consistent with this, engagement of PSGL-1 with specific mAbs that induce outside-in signaling (15) resulted in synthesis of the protein in protocols using both free and cross-linked antibodies (Fig. 1*b* and data not shown). Engagement of PSGL-1 by its natural ligand, P-selectin, was also sufficient for synthesis of the UPAR protein (Figs. 1*c* and 2). This contrasts with the requirement for a "second signal" for synthesis of gene products when β_1 integrins on monocytes are engaged (9). Synthesis of UPAR in monocytes adherent to immobilized P-selectin was rapid, with protein detectable by 30 min (Figs. 2 and 3), and was minimally triggered by incubation of the leukocytes on immobilized albumin, fibronectin, laminin, or collagen IV (Fig. 2*b* and data not shown), indicating selective

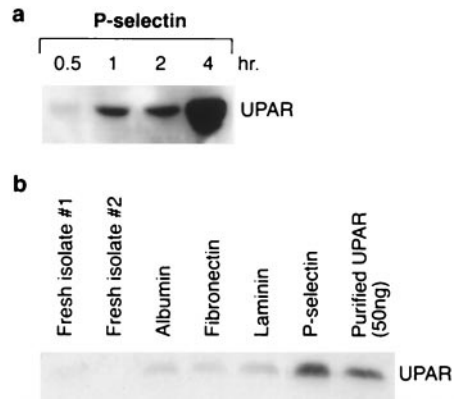

Fig. 2. UPAR is rapidly and differentially synthesized in adherent human monocytes. (*a*) Monocytes adherent to purified immobilized P-selectin were lysed after the times shown and probed for the presence of UPAR protein by Western analysis. A similar time course was seen in two additional experiments. (*b*) Monocytes were allowed to settle onto wells coated with immobilized proteins, incubated for 4 h, lysed, and immunoblotted for UPAR. Freshly isolated monocytes from two different subjects were immediately lysed and assayed in parallel. The experiment is representative of three others with similar results.

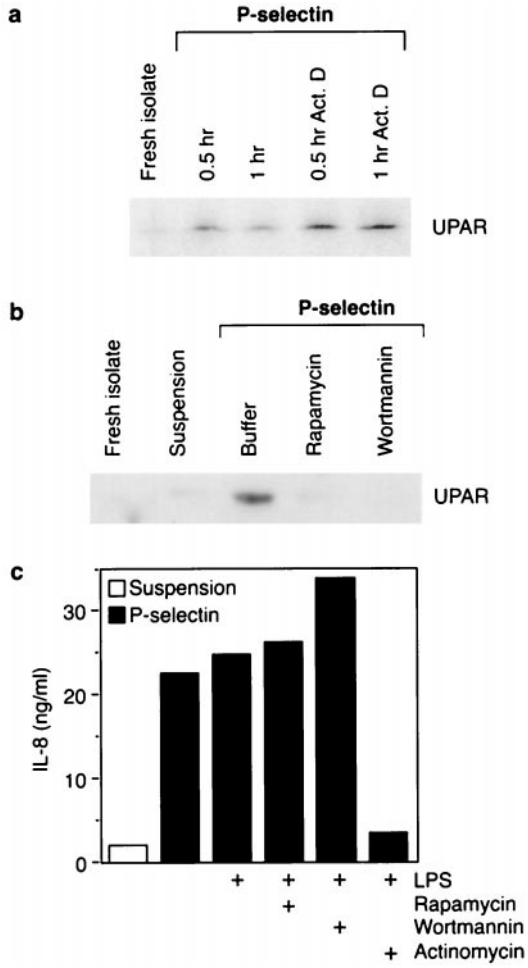

Fig. 3. Rapid UPAR synthesis does not require transcription in monocytes adherent to P-selectin and is regulated by mTOR. (*a*) Monocytes were allowed to adhere to P-selectin in the presence or absence of actinomycin D (10 μg/ml), and lysates were blotted for UPAR. Similar results were seen in two additional experiments. (*b*) Monocytes were lysed immediately after isolation (fresh isolate), were incubated in suspension, or were incubated while adherent to P-selectin (4 h) in the presence of rapamycin (100 nM), wortmannin (100 nM), or vehicle-containing buffer. The cells were then lysed and blotted for UPAR. Inhibition of UPAR synthesis by rapamycin and wortmannin was seen in three and five additional experiments, respectively. (*c*) Monocytes were incubated in suspension or adherent to P-selectin for 4 h. LPS, rapamycin, wortmannin, or actinomycin D were added to some replicates as indicated, and total IL-8 was measured in the lysates and supernates from each sample by ELISA. A second experiment yielded similar results.

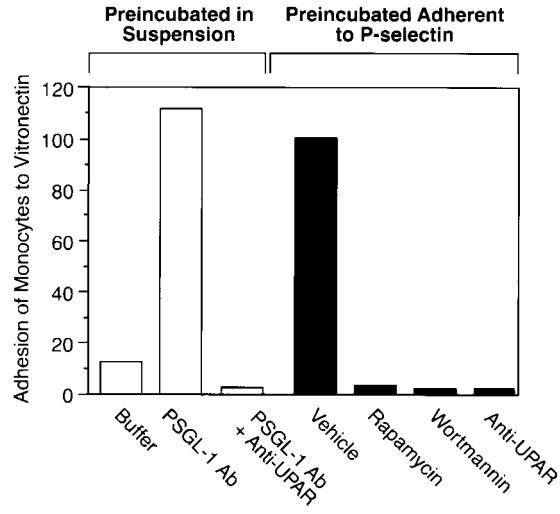

Fig. 4. Translationally regulated synthesis of UPAR mediates new recognition of vitronectin by monocytes. Monocytes were incubated in buffer alone or with mAb PL-2 (PSGL-1 Ab), which triggers UPAR expression (Fig. 1), transferred to wells precoated with vitronectin, and incubated for 1 h in the presence or absence of a blocking mAb against UPAR. In parallel, monocytes were preincubated while adherent to immobilized P-selectin in the presence of vehicle (DMSO), rapamycin (100 nM), or wortmannin (100 nM); they were then transferred to wells precoated with vitronectin, and adhesion was measured after a 1-h incubation in the presence or absence of the anti-UPAR mAb. The data are representative of six experiments in which the preincubation conditions were repeated a minimum of two times and inhibitor and mAb conditions a minimum of three times. See text for additional controls.

outside-in signaling. Incubation of monocytes on uncoated plastic resulted in low-level accumulation of UPAR protein that was much less than in cells adherent to P-selectin (Fig. 1), suggesting that the use of plastic adhesion in isolation protocols may account for previous reports that UPAR is constitutively present on monocytes (27, 28).

Rapid Synthesis of UPAR in Monocytes Adherent to P-Selectin Does Not Require Transcription and Is Regulated by mTOR. Although UPAR mRNA levels increased in monocytes adherent to P-selectin (Fig. 1 and data not shown), rapid expression of UPAR protein occurred in the presence of actinomycin D (Fig. 3*a*), indicating that transcription is not required. The synthesis of IL-8 was also triggered in monocytes adherent to P-selectin (Fig. 3*c*). This synthesis was variably enhanced by LPS (data not shown). In contrast to UPAR, however, its production was blocked by transcriptional inhibition with actinomycin D (Fig. 3*c*). These experiments demonstrate differential regulation of expression of two key gene products that are influenced by adhesion-dependent signals in monocytes.

To explore specialized translational control of UPAR synthesis, we first established that freshly isolated human monocytes contain mTOR by immunocytochemical analysis (not shown). We also found that they constitutively express eIF4E-binding proteins and p70S6 kinase (data not shown), which are critical downstream components of the pathway (3, 7, 8, 29). Rapamycin, a specific inhibitor and probe for mTOR (3, 7, 29–32), blocked synthesis of UPAR triggered by adhesion to P-selectin (Fig. 3*b*). Synthesis of UPAR also was blocked by an inhibitor of phosphatidylinositol-3-kinase, wortmannin (Fig. 3). Phosphatidylinositol-3-kinase lies upstream from mTOR in the signaling cascade from the plasma membrane, and its inhibition blocks the pathway (3, 7, 33). In contrast, neither rapamycin nor wortmannin blocked synthesis of IL-8 in monocytes adherent to P-selectin (Fig. 3), indicating specificity of their actions under the conditions of these experiments.

mTOR-Regulated Display of UPAR Influences Monocyte Surface Phenotype and Function. UPAR influences surface proteolysis, matrix interactions, and signaling and migration of monocytes and other leukocytes. A critical component of its multifactorial effects on leukocyte function is its recognition of the matrix protein vitronectin and adhesion mediated by this mechanism (23–25, 34–36). We found that monocytes preincubated in suspension adhered in low numbers to immobilized vitronectin (Fig. 4), consistent with the paucity of UPAR under basal conditions (Figs. 1–3). When PSGL-1 was engaged with an activating antibody or when the monocytes were first preincubated while adherent to immobilized P-selectin (conditions that induce synthesis of UPAR; Fig. 1), adhesion to vitronectin was dramatically increased (Fig. 4). This adhesive capacity was inhibited by

a blocking antibody against UPAR (Fig. 4) but not by mAbs against integrin $\alpha_v\beta_3$, which also recognizes vitronectin, or by EDTA, which interrupts integrin-mediated adhesion (data not shown). Treatment of monocytes with rapamycin or wortmannin during their preincubation while adherent to P-selectin dramatically inhibited their ability to subsequently adhere to vitronectin (Fig. 4 and data not shown), consistent with inhibition of synthesis of UPAR by these agents (Fig. 3*b*). Thus, blockade of the mTOR pathway prevents synthesis of UPAR and consequent recognition of vitronectin, identifying a mechanism of "cross-talk" between surface adhesion molecules in which engagement of one tethering factor transmits outside-in signals that regulate expression of another at translational checkpoints.

As a second assay of UPAR display triggered by engagement of PSGL-1, we examined binding of exogenously added UPA by FACS analysis and found that adhesion of monocytes to P-selectin for 4 h induced increased surface binding of FITC-labeled UPA when compared with cells incubated in suspension. This was completely inhibited by pretreatment of the monocytes with rapamycin (not shown), consistent with assays examining recognition of vitronectin (Fig. 4). Together, these experiments provide evidence that mTOR regulates rapid changes in surface phenotype of specialized cells, in addition to its well known roles in cell cycle progression and cell growth (3, 7). It is possible that this action may account for some of the beneficial effects of rapamycin in pathologic processes that are influenced by monocytes or other cells in which this mechanism may operate, such as angiogenesis and restenosis (37, 38).

The Translational Checkpoint eIF4E Regulates UPAR Synthesis and Is Phosphorylated in Monocytes Adherent to P-Selectin. eIF4E, the least abundant translation initiation factor in most cell types, binds to the mRNA cap and, together with a second initiation factor, eIF4G, assembles a translation initiation complex that includes a helicase that unwinds the leader sequences of transcripts with extensive 5′ UTR secondary structure (8, 29). One mechanism of control of eIF4E is via signaling to mTOR, which regulates phosphorylation of the inhibitory eIF4E-binding proteins (see above) (7, 8, 29, 31). A parallel mechanism that controls eIF4E activity is phosphorylation on a critical serine residue, which enhances eIF4E binding to the mRNA cap structure and its interaction with eIF4G (6, 8, 29). We found that eIF4E is phosphorylated when monocytes adhere to P-selectin (Fig. 5*a*). This event is rapidly induced, consistent with the time course of UPAR synthesis (Figs. 2 and 3) and with the kinetics of mitogen-activated protein kinase activation in myeloid leukocytes in response to PSGL-1 engagement (15). Thus, adhesion of monocytes to P-selectin mediates outside-in signaling to a second major checkpoint in translational control, phosphorylation of eIF4E.

Overexpression of eIF4E facilitates translation of some, but not all, mRNAs with inhibitory secondary structure in their 5′ UTRs, in part dependent on cell-specific factors (39–42). To explore the regulation of UPAR in myeloid cells, we generated stable transfectant U937 monocytic cells that expressed substantially greater amounts of eIF4E protein than did wild-type cells or cells transfected with control vectors (Fig. 5*b*). UPAR was dramatically increased in the eIF4E-overexpressing myeloid cells (Fig. 5*c*). In contrast, the levels of actin were similar in eIF4E-overexpressing and wild-type cells, consistent with a previous report that its synthesis is independent of translational control (43). In addition, IL-8 was not increased in lysates or supernates of the overexpressing cells (not shown), consistent with differential regulation of UPAR and IL-8 synthesis in monocytes and with translational regulation of UPAR (Fig. 3). Together, these experiments indicate that the UPAR mRNA is a downstream target of eIF4E. Identification of such differentially regulated transcripts is essential for understanding signal-dependent translational regulation (29).

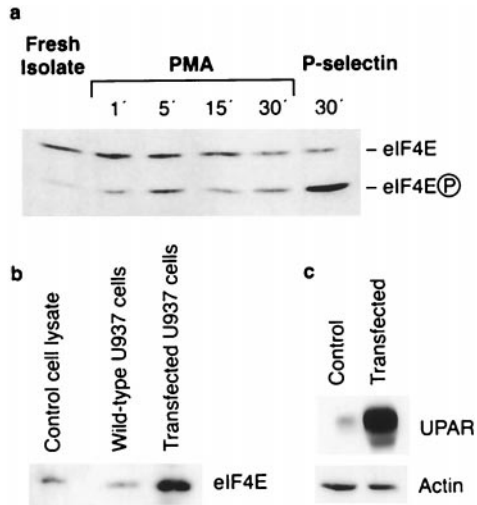

Fig. 5. Adhesion of monocytes to P-selectin induces phosphorylation of eIF4E, a regulator of UPAR synthesis. (*a*) Monocytes (5×10^6) were lysed immediately after isolation or were incubated for the indicated times in suspension in the presence of 100 nM phorbol 12-myristate 13-acetate or while adherent to immobilized P-selectin for 30 min. eIF4E was resolved by vertical slab isoelectric focusing and analyzed by immunoblotting. The figure is representative of three independent experiments. (*b*) U937 myelomonocytic leukocytes stably transfected with eIF4E or wild-type U937 cells were lysed, samples were normalized to equal amounts of protein, and eIF4E was separated by affinity purification using 7-methyl G Sepharose and SDS/PAGE and detected by immunoblotting. A lysate of control cells known to contain eIF4E was blotted in parallel. No induction of eIF4E occurred when U937 cells were transfected with an empty vector or with a vector containing the eIF4E cDNA subcloned in the opposite orientation (not shown). (*c*) U937 cells were harvested 20 days after transfection with eIF4E and lysed in parallel with control U937 cells; lysates were then immunoblotted with mAb against UPAR or actin. U937 cells harvested at longer periods after transfection with eIF4E also had dramatically increased levels of UPAR (not shown).

Adhesion, Signal-Dependent Translation, and Gene Expression in Myeloid Leukocytes. Our studies demonstrate that signal-dependent translational control of expression of specific gene products occurs in myeloid leukocytes and that engagement of PSGL-1 signals to mTOR and eIF4E in these cells. We also show that mTOR can regulate rapid changes in surface phenotype of specialized effector cells, in addition to controlling cell growth (3, 7). The latter observation points to broader roles for the mTOR pathway in physiologic and pathologic processes than currently recognized. We recently found that integrins on human platelets deliver outside-in signals to an mTOR-dependent translational control pathway, consistent with this possibility (16, 17). Adhesion-dependent translation of constitutive mRNAs via specific signaling pathways without a requirement for transcription, as we show for UPAR, has biologic advantages for myeloid leukocytes. These cells are specialized to circulate in a quiescent state but to undergo rapid phenotypic changes in a spatially regulated manner at targeted sites of inflammation or wound surveillance and repair (9, 13). Signal-dependent translation of constitutive transcripts that code for critical factors provides such a rapid response mechanism. In addition to rapidity, signal-dependent translational control provides precision in the synthesis of potent gene products with critical actions. Translational control can operate alone, or in concert with, transcriptional and other posttranscriptional mechanisms (4, 5, 26). UPAR, because of its potent and diverse biologic actions (23–25), is a

prime candidate for control at multiple checkpoints. In lymphocytes, outside-in signals from integrins alter stability of UPAR mRNA (44), suggesting that this posttranscriptional mechanism may also be in the repertoire of adherent monocytes (Fig. 1). Nevertheless, signal-dependent translational control clearly regulates rapid synthesis of UPAR in monocytes adherent to P-selectin (Figs. 3–5) and is likely to be a general mechanism of regulation of critical products in stimulated cells (16, 17, 45). Additional experiments indicate that other mRNAs (Table 1) are handled by mTOR in adherent monocytes (T.S.M., G.A.Z., unpublished experiments) and in other myeloid leukocytes (S. Lindemann, A.S.W., G.A.Z., unpublished results).

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