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Adherence-dependent Increase in Human Monocyte PDGF(B) mRNA Is Associated with Increases in c-*fos*, c-*jun*, and EGR2 mRNA

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Abstract. Adherence is an important initial step in the transition of a circulating monocyte to a tissue macrophage. This differentiation is accompanied by an augmented capacity to generate growth factors. We hypothesized that adherence itself might be an important trigger for a sequence of gene activation culminating in cells with increased mRNA encoding profibrotic growth factors such as platelet-derived growth factor B subunit (PDGF[B]) and transforming growth factor-β (TGF-β). After in vitro adherence, human monocytes had a biphasic increase in PDGF(B) mRNA with peaks at 6 h and 13 d. No increase in TGF-β mRNA was observed. The 6-h increase in PDGF(B) mRNA was adherence dependent, and in addition, was abrogated when the cytoskeletal integrity was compromised by cytochalasin D. The 6-h increase in PDGF(B) mRNA was unaltered by adherence in the presence of the monocyte stimulus lipopolysaccharide. Adherence to either fibronectin or collagen-coated plastic had little consistent effect on PDGF(B) mRNA accumulation. The increased PDGF(B) mRNA observed in adherent monocytes was accompanied by increases in mRNAs of the early growth response genes c-*fos* (maximal at 20 min), c-*jun*, and EGR2 (maximal at 6–24 h). The increase in c-*jun* and EGR2, but not c-*fos*, mRNA was also abrogated by cytochalasin D. These observations suggest that adherence results in increases of c-*fos*, c-*jun*, EGR2, and PDGF(B) mRNA. In addition, the increases in c-*jun*, EGR2, and PDGF(B) may depend on cytoskeletal rearrangement. Modulation of these events at the time of adherence offers a mechanism by which differential priming of the cells may be accomplished.

ADHERENCE to endothelium and then extracellular matrix is a prerequisite for peripheral blood monocyte migration into injured tissues. Here the monocyte undergoes differentiation into a tissue macrophage. Monocyte adherence results in activation of the genes c-*fos*, TNF-α, and CSF-1, but not HLA-DR-α (Haskill et al., 1988). Thus, adherence may initiate a macrophage differentiation pathway by priming macrophages for later cytokine production.

The macrophage is believed to play an important role in orchestrating the fibrotic response as it occurs in wound healing or in the pathological circumstances of pulmonary fibrosis (Crystal et al., 1984; Rappolee et al., 1988). For the present studies we have focused on two profibrotic cytokines produced by macrophages: PDGF and transforming growth factor β (TGF-β)[1] (Shimokado et al., 1985; Assoian et al., 1987).

PDGF is a major profibrotic cytokine which stimulates fibroblast proliferation, chemotaxis, and contraction. PDGF can be a homodimer of A chains or B chains or a heterodimer of A and B chains; however, fibroblasts appear to have more receptors for molecules containing the B chain (Hosang et al., 1989). Therefore, molecules containing the B chain are the more potent fibroblast mitogens (Beckman et al., 1988), chemoattractants (Nister et al., 1988), and agonists for fibroblast-mediated contraction of collagen gels (Clark et al., 1989). Recombinant PDGF(B) homodimer has also been shown to promote wound healing in vivo (Pierce et al., 1988). The B subunit (PDGF[B]) is encoded by the c-*sis* protooncogene, and the mRNA is expressed in human alveolar macrophages, in macrophages derived from monocytes cultured in vitro for 10–14 d (Mornex et al., 1986), and after differentiation of human cell lines to macrophage-like cells (Pantazis et al., 1986). The alveolar macrophage PDGF(B) mRNA is elevated in some patients with interstitial lung disease (Shaw, R. J., R. A. F. Clark, S. H. Benedict, and T. E. King Jr., manuscript submitted for publication) along with an increased PDGF production (Martinet et al., 1987). Similarly, in systemic sclerosis, the skin macrophages have increased PDGF(B) mRNA (Olsen and Uitto, 1989) and increased cytoplasmic PDGF protein (Gay et al., 1989).

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1. *Abbreviations used in this paper*: (B), B subunit; Fn, fibronectin; LPS, lipopolysaccharide; TGF-β, transforming growth factor β.

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TGF-β promotes wound repair in rat model systems (Sporn et al., 1983; Lawrence et al., 1986; Mustoe et al., 1987) and induces fibrosis when injected into newborn mice (Roberts et al., 1986). The potential role of TGF-β as an endogenous mediator of fibroplasia is further supported by in vitro studies showing that the purified growth factor stimulates collagen and fibronectin production in cultures of some mesenchymal cells including dermal (Roberts et al., 1986; Ignotz and Massague, 1986) and lung (Fine and Goldstein, 1987; Raghu et al., 1989) fibroblasts.

In previous studies on macrophage differentiation of the human promyelocytic cell HL60 (Shaw, R. J., R. A. F. Clark, V. P. Sukhatme, A. G. Ritter, P. A. Campbell, and S. H. Benedict, manuscript submitted for publication), we found evidence that HLA-DR-α and PDGF(B) genes were not coordinately regulated, and that increases in PDGF(B) mRNA were preceded by increases in c-*fos*, c-*jun*, and EGR2 mRNAs, which encode proteins that may potentially regulate gene activation (Benedict and Chan, 1990). Haskill et al. (1988) found that adherence increased c-*fos* but not HLA-DR-α mRNA. Human monocytes differentiate in vitro in the presence of serum to macrophages (Musson, 1983), and we used this system to ask whether PDGF(B) mRNA accumulation occurred during the early stages of macrophage differentiation, and whether it was associated with increases in c-*jun* and EGR2 mRNAs as well as c-*fos* mRNA. Our results suggest that monocyte to macrophage differentiation involves a series of events including adherence, c-*fos* gene activation, cytoskeletal rearrangement, and c-*jun*, EGR2, and PDGF(B) mRNA accumulation.

Materials and Methods

Isolation of Monocytes

Human peripheral blood monocytes (>92% pure, >85% yield) were isolated by a combination of plasma-Percoll density gradients (Haslett et al., 1985) and counterflow centrifugation–cell elutriation in the absence of an adherence step using a J2-21 centrifuge equipped with a stroke rpm JE-6B elutriator rotor (Beckman Instruments, Inc., Fullerton, CA) (Doherty et al., 1987). This system was sterile and without significant lipopolysaccharide (LPS) contamination. It utilized Krebs-Ringer phosphate buffer, pH 7.2, made from salts purchased from Malinckrodt Inc. (St. Louis, MO) supplemented with 0.2% dextrose (diluted from 0.5% dextrose in 0.2% sodium chloride; Abbott Laboratories, Irving, TX). Cells were suspended in medium 199 (Gibco Laboratories, Grand Island, NY) supplemented with 5% autologous serum, glutamine, penicillin, and streptomycin (Irvine Scientific, Santa Ana, CA), and allowed to adhere at 37°C in 100-mm tissue culture plates (Becton Dickinson, Lincoln Park, NJ). In some experiments dishes were coated with collagen (Collagen Corp., Palo Alto, CA) or fibronectin (New York Blood Center, New York) with nonspecific binding sites blocked by the further addition of LPS-free human serum albumin (Calbiochem-Behring Corp., San Diego, CA). LPS from *Escherichia coli* 0111:B4 (List Biologicals, CA) and cytochalasin D (Sigma Chemical Co., St. Louis, MO) were added, as indicated, to the culture at the same time as the cells. Nonadherent cells were maintained in rotating 50-ml polypropylene tubes (Becton Dickinson) at 37°C.

Since these studies examined the effects of LPS on gene activation in monocytes, scrupulous attention was paid to avoiding LPS contamination of the cells during preparation and culture. Accordingly, all reagents were tested by the Limulus Amoebocyte assay kit (Associates of Cape Cod, Woods Hole, MA), and at the concentrations of reagents used in these experiments the final culture and stimulation mixtures contained <0.01 ng/ml LPS.

Isolation of RNA

The adherent cells were lysed by suspension in guanidinium isothiocyanate solution, containing 4 M guanidinium isothiocyanate (Boehringer Mannheim Biochemicals, Indianapolis, IN), 25 mM sodium citrate, pH 7.0, 17 mM sodium *N*-laurylsarcosine, 0.14 M 2-mercaptoethanol (Sigma Chemical Co.). The RNA was separated by centrifugation through a 5.7 M cesium chloride (Bethesda Research Laboratories, Gaithersburg, MD) cushion at 35,000 rpm overnight using an L8-80 ultracentrifuge and SW-55 rotor (Beckman Instruments, Inc.) (Glisin et al., 1974; Chirgwin et al., 1979). The pellet was washed in 70% ethanol and the RNA suspended in diethyl pyrocarbonate (Sigma Chemical Co.)-treated water, further extracted with phenol/chloroform, resuspended in 0.15 M Na acetate, and stored at −20°C in 70% ethanol. The RNA was harvested by centrifugation (12,000 *g*) at 4°C for 30 min.

RNA Gel (Northern) Analysis

The RNA was suspended in sample buffer (65% deionized formamide, 15% formaldehyde, 0.05 M 3-[*N*-morpholino] propanesulfonic acid [Sigma Chemical Co.]). RNA (15 μg/well) was electrophoresed through an agarose/formaldehyde gel (Maniatis et al., 1982) and blotted onto a nylon membrane (Zeta-Probe; Bio-Rad Laboratories, Richmond, CA), which was then baked at 80°C for 2 h. In all cases, the amounts of RNA loaded were shown to be the same in each experiment by staining the gel with ethidium bromide and photography under UV light.

Before hybridization, the membranes were prehybridized at 42°C for 24 h in 10× Denhardt's solution (0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% BSA), 5× SSC (0.75 M sodium chloride, 0.075 M sodium citrate), 50 mM Na_2HPO_4, 2% SDS, 45% formamide, and 0.5 mg/ml salmon sperm DNA. Blots were then hybridized at 42°C for 24 h, with probes labeled by the random primer procedure using (α-^{32}P) dCTP (Amersham Corp., Arlington Heights, IL) (Feinberg and Vogelstein, 1983). Specific activities of probes were ~10^9 cpm/μg DNA. The membranes were washed four times with 2× SSC, 0.1% SDS at 22°C, followed by two washes with 0.2× SSC, 0.1% SDS at 65°C for 30 min, before autoradiography with exposures of 18 (c-*fos*, c-*jun*, and EGR2) and 72 h (PDGF[B]).

The relative intensity of the bands was compared by scanning densitometry using a DU-65 spectrophotometer with Gelscan program (Beckman Instruments, Inc.).

To eliminate any effect of differences in cells from different donors or in hybridization efficiency between experiments, all experiments were related to an internal control which was the mRNA abundance 6 h after adherence for all genes except c-*fos* when the 20-min post-adherence value was chosen. These points were included in all experiments and ascribed the 100% value.

DNA Probes

The human DNA probes used were a 0.75-kb Eco RI c-*sis* fragment (gift of M. Murray, ZymoGenetics, Seattle, WA), a 1.65-kb Eco RI TGF-β fragment (gift of A. Purchio, Oncogen, Seattle, WA), a 1.9-kb Nae I c-*fos* fragment (ATCC 41046), a 1.5-kb Hind III–Eco RI c-*jun* fragment (gift of M. Karin, University of California, San Diego, CA), a 0.6-kb Hind III–Eco RI EGR2 fragment (gift of V. P. Sukhatme, University of Chicago, Chicago, IL), and a 3.1-kb Eco RI HLA-DR α fragment (gift of S. Weissman, Yale University, New Haven, CT).

In all cases, probe specificity was high, with the approximate sizes of the mRNA, as assessed in relation to the 28S (4.7-kb) and 18S (1.9-kb) ribosomal RNA, of 3.8 kb for PDGF(B), 2.4 kb for TGF-β, 3.5 kb for EGR2, 3.0 kb for c-*jun*, 2.2 kb for c-*fos*, and 0.76 kb for HLA-DR-α.

Results

Monocyte Adherence Was Accompanied by an Increase in PDGF(B), but Not TGF-β mRNA

Fresh peripheral blood monocytes had little or no constitutive PDGF(B) mRNA, but upon incubation under adherent conditions had a biphasic increase in PDGF(B) mRNA (Fig. 1 *a*). Pooled data from multiple experiments were compared and expressed as percent of the 6-h post-adherence values. The biphasic increase in PDGF(B) mRNA commenced at 1 h, was maximal at 6 h, decreased by 24 h, and then gradually increased again to 50% of the 6-h value by 13 d. Fresh peripheral blood monocytes had a substantial but variable amount of TGF-β mRNA, which tended to decrease when monocytes were cultured on plastic (Fig. 1 *b*). Data were

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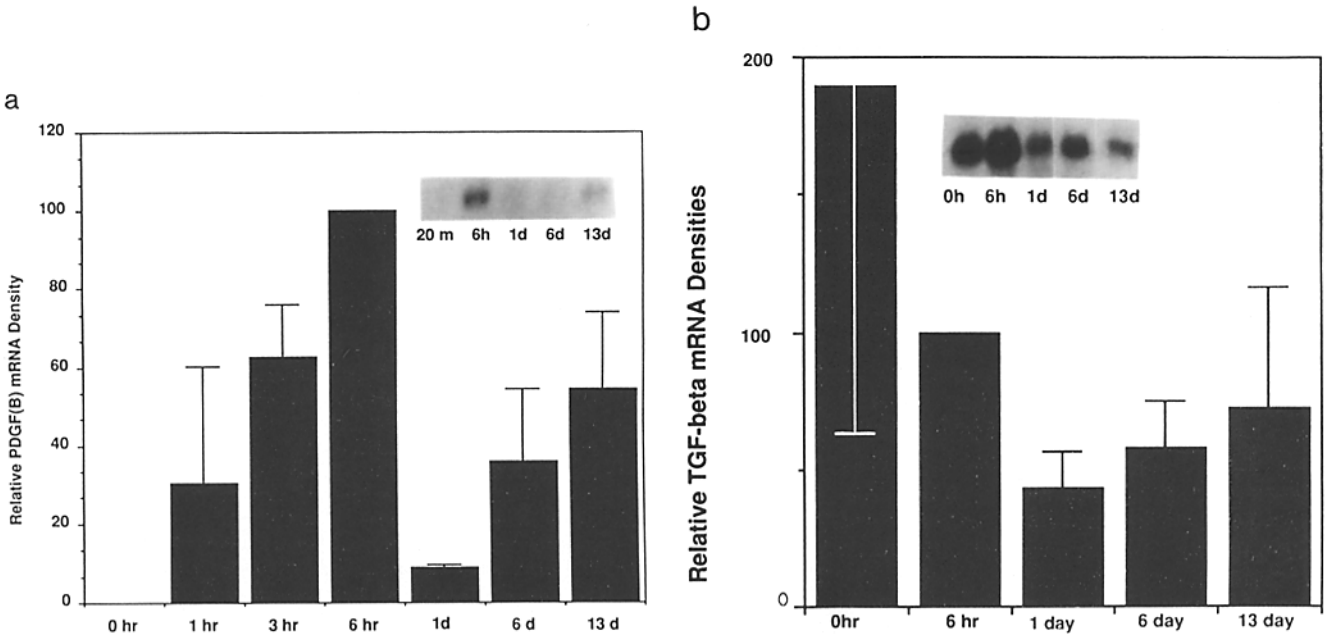

Figure 1. Kinetics of growth factor mRNA increase in adherent monocytes. (*a*) PDGF(B) mRNA; (*b*) TGF-β mRNA. Quantification of mRNA was obtained by densitometry of autoradiograms of RNA blots. mRNA densities are expressed as a percent of mRNA values at 6 h ($n = 3$). (*Insets*) Autoradiograms of two blots of RNA from monocytes 0 (*a*) or 20 min (*b*), and 6 h, and 1, 6, and 13 d after culture under adherent conditions.

again pooled and expressed as a percent of the 6-h culture values. In all subsequent experiments, the PDGF(B) or TGF-β mRNA values of monocytes cultured on plastic for 6 h were included as a reference point to which mRNA values of monocytes under other conditions were compared.

Adherence Dependence of the 6-h Increase in PDGF(B) mRNA

Experiments were performed to determine whether the 6-h increase in PDGF(B) mRNA was due to adherence or to ex-

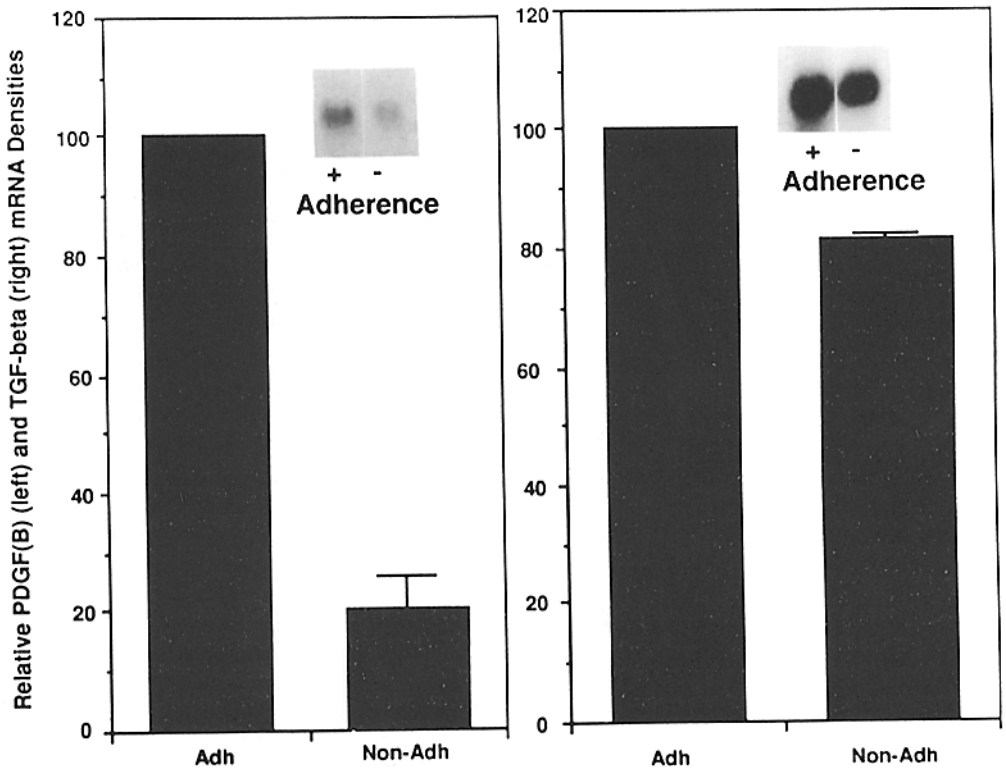

Figure 2. Relative abundance of growth factor mRNA in monocytes cultured under adherent or nonadherent conditions. (*Left*) PDGF(B) mRNA; (*right*) TGF-β mRNA. mRNA densities are expressed as a percent of values from cells incubated for 6 h on plastic ($n = 6$). (*Insets*) Autoradiograms of Northern blots of RNA from monocytes 6 h after culture under adherent or nonadherent conditions.

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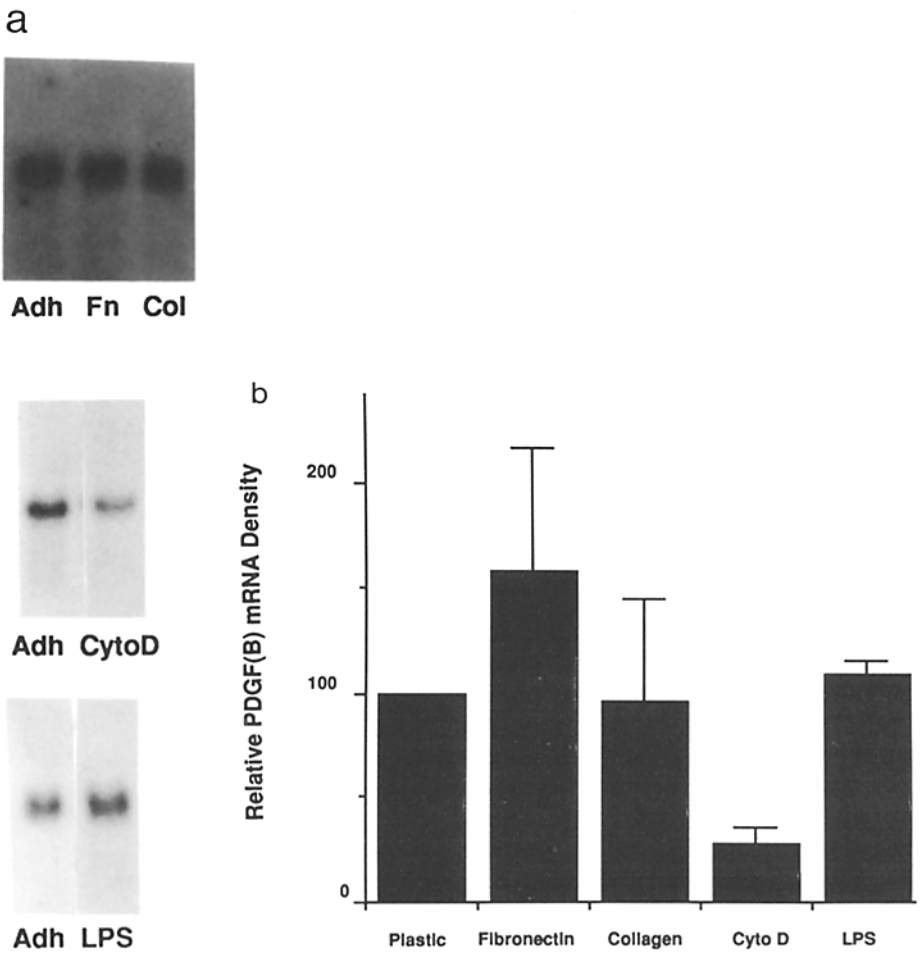

Figure 3. Effect of adherence, cytoskeletal integrity, and LPS stimulation on PDGF(B) mRNA levels in monocytes. Monocytes were cultured for 6 h on tissue culture plastic (*Adh*), fibronectin (*Fn*), or collagen-coated (*Col*) plastic, or on plastic in the presence of 2 μg/ml cytochalasin D (*CytoD*) or 1 μg/ml lipopolysaccharide (*LPS*). (*a*) Representative autoradiograms of Northern blots. (*b*) PDGF(B) mRNA abundance obtained by densitometry of autoradiograms and expressed as percent of mRNA values of cells incubated for 6 h on plastic ($n \geq 3$).

posure to the serum containing medium. The inset in the left panel of Fig. 2 shows that the early (6-h) increase in PDGF(B) mRNA was dependent on adherence, as it was not observed in cells incubated for 6 h in the same medium but under nonadherent conditions. Furthermore, when pooled data from six experiments was compared, monocytes incubated under nonadherent conditions had only 21% of the PDGF(B) mRNA of cells cultured under adherent conditions (Fig. 2, *left*). Thus, the first phase of the biphasic increase in PDGF(B) mRNA in monocytes undergoing differentiation to macrophages was dependent on adherence. In contrast, the relative amount of TGF-β mRNA observed when isolated monocytes were cultured on plastic was not substantially altered when monocytes were incubated under nonadherent conditions (Fig. 2, *right*).

Modulation of the Adherence-dependent Increase in PDGF(B) mRNA

The adherence-dependent increase in monocyte PDGF(B) mRNA was diminished threefold when cytochalasin D (2 μg/ml), an inhibitor of cytoskeletal rearrangement, was present during the 6-h culture under adherent conditions (Fig. 3, *a* and *b*). The cytochalasin D did not alter the number of cells adhering to the plastic (Table I).

Adherence to plastic previously coated with fibronectin (Fn) resulted in a small and variable increase of <50% in PDGF(B) mRNA when compared to adherence to plastic alone (Fig. 3, *a* and *b*). Adherence to collagen-coated plastic or to noncoated plastic in the presence of 1 μg/ml of LPS did not alter the adherence-dependent increase in PDGF(B) mRNA observed at 6 h. Fn and collagen-coated plastic as well as LPS caused a modest increase in the percent of adherent monocytes (Table I).

mRNAs of Early Growth Response Genes Increase during Monocyte Adherence and Differentiation

Many early growth response proteins bind DNA and regulate gene expression. It was of interest to examine whether certain early growth response mRNAs accumulated in association with adherence stimulated PDGF(B) mRNA. Immediately after elutriation and before culture, there was variable constitutive expression of c-*fos* and c-*jun* but not EGR2 mRNA (data not shown). During monocyte adherence in culture there were initially high levels of c-*fos* mRNA maxi-

Table I. Monocyte Adherence

Plastic*	Cytochalasin D	Fn	Collagen	LPS
62.7(3.0)	66.4(9.4)	86.5(3.6)	85.3(4.7)	80.5(6.7)

* Percent adherence of monocytes after a 6-h incubation in tissue culture wells alone (*Plastic*), or with cytochalasin D (2 μg/ml), fibronectin-coated plastic (*Fn*), collagen-coated plastic (*Collagen*), or LPS (1 μg/ml). Mean (SEM); $n = 4$.

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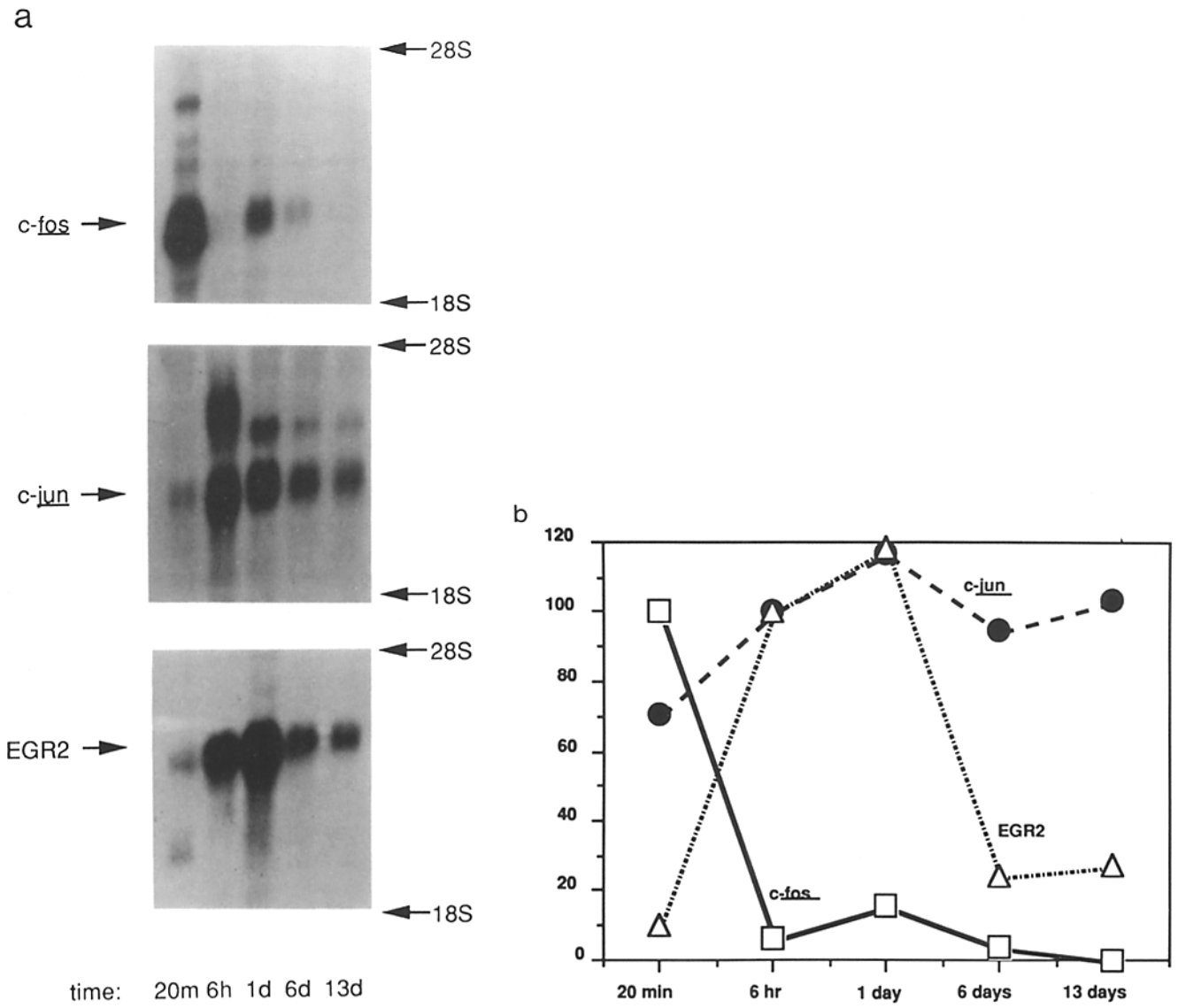

Figure 4. Time course of increase in c-*fos*, c-*jun*, and EGR2 mRNA in monocytes during adherence. (*a*) Representative autoradiograms of Northern blots of RNA from monocytes at 20 min, 6 h, and 1, 6, and 13 d after culture under adherent conditions as in Fig. 1. (*b*) Time course of changes in c-*fos*, c-*jun*, and EGR2 mRNA in monocytes during in vitro differentiation to macrophages over 13 d. Results are expressed as a percent of 20-min values for c-*fos* and 6-h values for c-*jun* and EGR2 (n = 3).

mal at 20 min, which decreased at 6 h, increased slightly at 24 h, and then declined at 6 and 13 d (Fig. 4, *a* and *b*). There were variable constitutive levels of c-*jun* mRNA in monocytes. After adherence in culture there was an increase in c-*jun* mRNA expression as early as 20 min in some experiments but consistently by 6 h to levels sustained at 24 h. The abundance of c-*jun* mRNA at 6 and 13 d was also variable but was in general sustained at high levels. EGR2 mRNA was constitutively present at very low levels in monocytes, but was increased at 6 h with a maximum at 24 h, and declined to levels slightly above background at 6 and 13 d.

Dependence of c-fos mRNA Increase on Adherence

At the end of the elutriation, when the monocytes were suspended in Krebs-Ringer phosphate buffer, the cells already contained high levels of c-*fos* mRNA (Fig. 5 *a*, *first lane*). When the cells were suspended in culture medium containing autologous serum, and incubated for 20 min, there were high levels of c-*fos* when cultured under adherent conditions (Fig. 5 *a*, *second lane*), but low levels when cultured under nonadherent conditions (Fig. 5 *a*, *third lane*). The high levels of c-*fos* mRNA observed under adherent conditions at 20 min had declined by 60 min (Fig. 5 *a*, *fourth lane*). Thus adherence induced a brief but intense increase in the level of c-*fos* mRNA. This was confirmed when cells were incubated under nonadherent conditions for 6 h and then divided such that one aliquot was allowed to adhere for 20 min, while the other aliquot was maintained under nonadherent conditions for an additional 20 min. In these experiments cells were also found to be capable of an increase in c-*fos* mRNA in response to adherence. Cells cultured under continued nonadherent conditions showed no such increase (Fig. 5 *b*).

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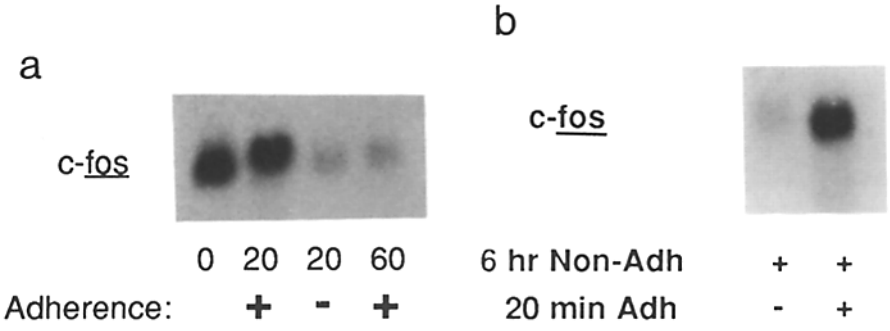

Figure 5. Effect of adherence on c-*fos* mRNA in monocytes. (*a*) Representative autoradiogram of Northern blot of RNA from monocytes in Krebs-Ringer phosphate buffer immediately after elutriation (*first lane*), adherence for 20 min (*second lane*), suspension in medium for 20 min (*third lane*), and adherence for 60 min (*fourth lane*). (*b*) Representative autoradiogram of Northern blot of RNA from monocytes cultured under nonadherent conditions for 6 h followed by adherence for 20 min, or further incubation under nonadherent conditions for 20 min and hybridized for c-*fos* mRNA ($n = 4$).

Relationship of c-fos, c-jun, and EGR2 mRNA Increase to Adherence and Cytoskeletal Integrity

When monocytes were cultured under nonadherent conditions for 6 h and c-*jun* and EGR2 mRNA levels were compared to the levels in monocytes cultured under adherent conditions, two- and fourfold reductions in c-*jun* and EGR2 mRNA, respectively, were observed (Fig. 6 *a*). The pooled results of several experiments are shown in Fig. 6 *b*. In these experiments nonadherent monocytes cultured for 20 min, again, contained lower levels of c-*fos* mRNA than did adherent cultures.

To examine the effect of cytoskeletal integrity on c-*fos*, c-*jun*, and EGR2 RNA levels, cytochalasin D was added to monocytes incubated under adherent conditions for 20 min and probed for c-*fos* or for 6 h and probed for c-*jun* and EGR2. Cytochalasin D did not affect the increase in c-*fos* mRNA observed 20 min after culture under adherent conditions (Fig. 6, *a* and *b*). However, adherence for 6 h in the

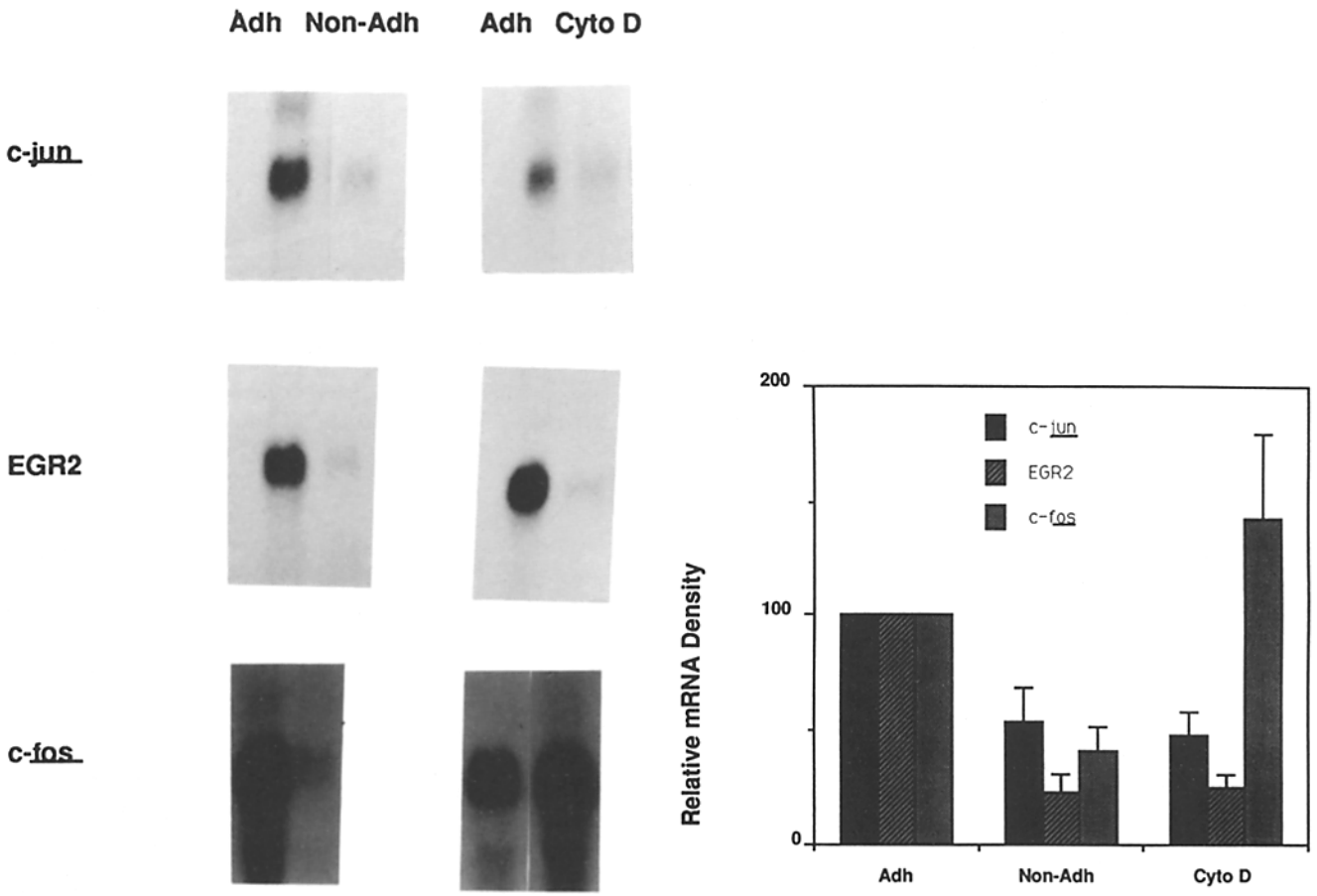

Figure 6. Effect of adherence and cytoskeletal changes on c-*fos*, c-*jun*, and EGR2 mRNA in monocytes. Relative abundance of c-*fos*, c-*jun*, and EGR2 mRNA incubated under adherent conditions (*Adh*), nonadherent conditions (*Non-Adh*), or adherent conditions in the presence of 2 μg/ml cytochalasin D (*Cyto D*), for 20 min in the case of c-*fos*, and for 6 h in the case of c-*jun* and EGR2. (*a*) Representative autoradiograms. (*b*) mRNA abundance expressed as percent of mRNA abundance at 20 min for c-*fos* and 6 h for c-*jun* and EGR2 ($n \geq 4$).

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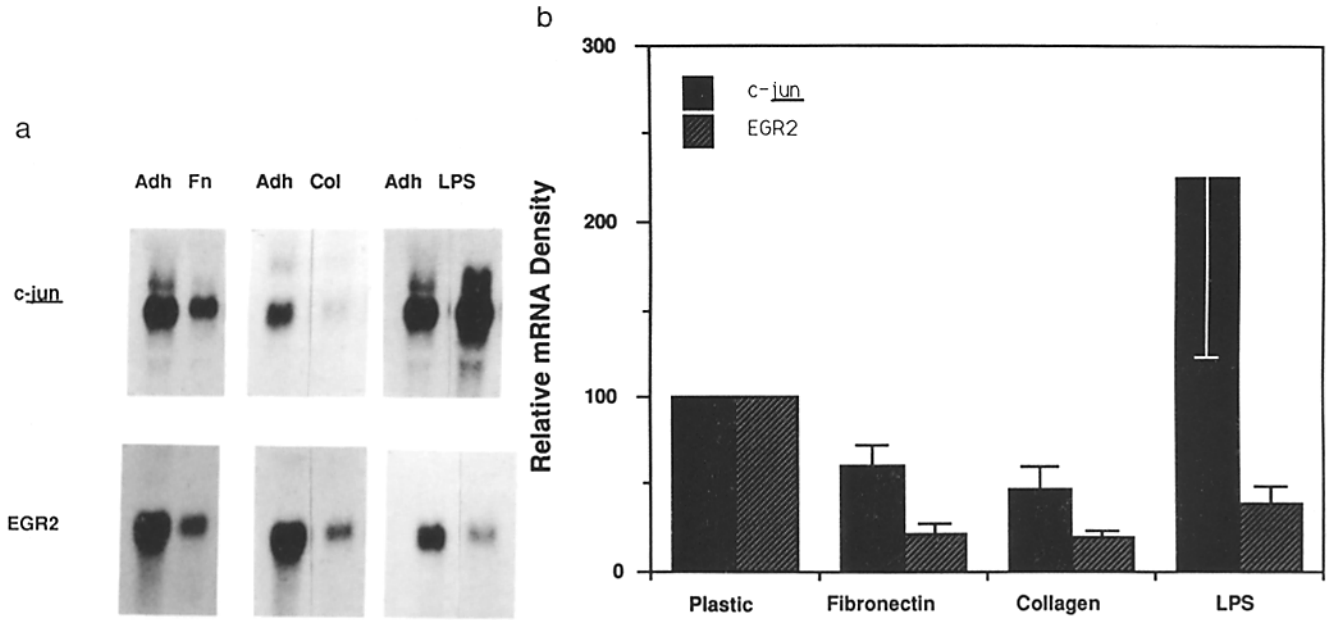

Figure 7. Modulation of c-*jun* and EGR2 mRNA in adherent monocytes. Relative abundance of c-*jun* and EGR2 mRNA in monocytes cultured for 6 h on tissue culture plastic (*Adh*), fibronectin (*Fn*) or collagen (*Col*)-coated plastic, or noncoated plastic in the presence of LPS (1 μg/ml). (*a*) Representative autoradiograms. (*b*) Data expressed as percent of values observed after 6 h of adherence on tissue culture plastic ($n \geq 3$).

presence of cytochalasin D diminished the increase of c-*jun* and EGR2 mRNA by two- and fourfold, respectively.

Relationship of c-jun and EGR2 mRNA Increase to Receptor Stimulation

We next determined whether the abundance of c-*jun* and EGR2 mRNA was modulated by Fn, collagen, or LPS. When cells adhered to Fn or collagen-coated plastic, the abundance of c-*jun* was only 60 and 48%, respectively, of that observed when cells adhered to plastic alone (Fig. 7, *a* and *b*). EGR2 mRNA in cells adhering to Fn and collagen-coated plates was 22 and 20% of that observed during adherence to plastic alone (i.e., a four- and fivefold reduction). Adherence in the presence of LPS increased the abundance of c-*jun* mRNA, but decreased EGR2 mRNA abundance. Thus c-*jun*, EGR2, and PDGF(B) genes were apparently not under coordinate control in the early adherence-dependent stages of monocyte differentiation to macrophages.

HLA-DR-α mRNA Was Unaffected by Adherence

The abundance of HLA-DR-α mRNA served as a control for the effects of adherence, and similar levels were observed in monocytes cultured for 6 h under adherent conditions alone, in the presence of cytochalasin D, or under nonadherent conditions (Fig. 8). Thus, the changes in PDGF(B), c-*fos*, c-*jun*, and EGR2 mRNA were not reflected by all other mRNA species.

Discussion

Biphasic Increase in PDGF(B) mRNA during Differentiation of Monocytes to Macrophages

The presence of PDGF(B) mRNA is indicative of the cell's commitment to the first stage of growth factor production. Accumulation of PDGF(B) mRNA renders the cell primed to respond to subsequent triggers by completing translation and secretion of PDGF in a similar manner to that described for TNF-α (Haskill et al., 1988). Such multiple levels of control are suggested by the observation that PDGF(B) mRNA is not directly correlated with the release of PDGF protein (Durga Rao et al, 1988). The biphasic increase in PDGF(B) mRNA in monocytes as they differentiate to macrophages indicates the complexity of this priming process (Fig. 1). To further underscore the complexity of the situation, TGF-β mRNA is expressed constitutively in circulating monocytes suggesting that monocyte TGF-β activity is primarily controlled at the translational or posttranslational level (Lyons et al., 1988; Miyazono and Heldin, 1989).

The timing of the biphasic increase in PDGF(B) mRNA in differentiating monocytes with maximal increases at 6 h and 13 d, roughly correlates with the increases in fibroblast activity in wounds (Welch et al., 1990). Fibroblast proliferation and influx, which require fibroblast mitogenic and chemotactic factors such as PDGF(B), occur during the first few days after injury, whereas wound contraction, mediated at least in part by PDGF(B) (Clark et al., 1989) occurs during the second week after injury. Our experiments do not distinguish between a biphasic response within all the cells or the possibility of two responding populations.

The present study demonstrated that the initial increase in PDGF(B) mRNA was dependent on adherence (Fig. 2), was inhibited by cytochalasin D (Fig. 3), and was accompanied by increases in the mRNA of the early growth response genes c-*fos*, c-*jun*, and EGR2 (see Fig. 4). Cytochalasin D at doses sufficient to disrupt cytoskeletal integrity (Schliwa, 1982) had no effect on the c-*fos* mRNA accumulation while at the same time c-*jun*, EGR2, and PDGF(B) mRNA were reduced by as much as 75%. These data, along with data from previ-

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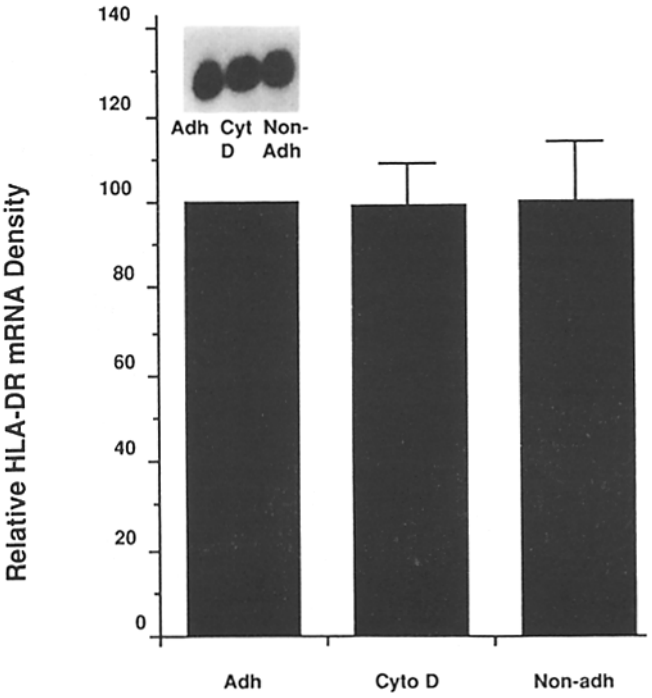

Figure 8. Relative abundance of HLA-DR-α mRNA in monocytes incubated for 6 h under adherent conditions alone (*Adh*), in the presence of 2 μg/ml cytochalasin D (*Cyto D*), or under nonadherent conditions (*Non-Adh*). Data are expressed as a percent of the 6-h adherence value ($n \geq 4$). (*Inset*) Representative autoradiogram of Northern blot of RNA from monocytes cultured as above and hybridized for HLA-DR-α.

ous studies in which c-*fos*, EGR2, c-*jun*, and PDGF(B) mRNAs are increased during differentiation of HL60 cells to macrophage-like cells (Shaw, R. J., R. A. F. Clark, V. P. Sukhatme, A. G. Ritter, P. A. Campbell, and S. H. Benedict, manuscript submitted for publication), lead to the speculation that a specific pattern of gene activation occurs during the early phase of monocyte differentiation. In this scenario, adherence would be followed by c-*fos* mRNA accumulation, cytoskeletal rearrangement, and later by increases in c-*jun*, EGR2, and PDGF(B) mRNA.

Interrelationship between Adherence, Cytoskeletal Integrity, and mRNA Accumulation

The sequence of events just described suggests that physical changes to the cell may either initiate gene activation or cause stabilization of preformed mRNAs. The precedent for adherence-dependent mRNA accumulation in monocytes was established for c-*fos*, CSF-1, and TNF-α by Haskill et al. (1988). Dependence of mRNA levels on cytoskeletal integrity has not been previously observed in monocytes but a relationship between the cytoskeleton and mRNA accumulation is recognized in other cell systems (for reviews see Ben-Ze'ev, 1987; Bissel and Barcellas-Hoff, 1987).

The first PDGF(B) mRNA increase was maximal at 6 h. This coincided with a time when the cells had completed adherence (Horsburgh et al., 1987), but before spreading of the cells with the development of cytoplasmic protrusions (our unpublished observations). More complete reorganization of the cytoskeleton elements occurs during the transition of monocytes to macrophages over the course of one week (Lehto et al., 1982). The role of these later morphological changes in modulating PDGF(B) mRNA accumulation is the subject of further study. It is also possible that PDGF has an autocrine effect altering cytoskeletal elements as occurs in fibroblasts (Herman and Pledger, 1985; Nister et al., 1988).

The early (6-h) rise in PDGF(B) mRNA was a direct consequence of adherence and dependent on cytoskeletal integrity (Figs. 2 and 3). Although the enhancement in the presence of Fn was only modest (Fig. 3 and Table I), Fn has been previously reported to increase monocyte adherence (Bevilacqua et al., 1981; Horsburgh et al., 1987) and to induce collagenase and stromelysin gene expression in fibroblasts (Werb et al., 1989). It was largely unaffected, however, by concurrent stimulation of the cells by collagen or LPS (Fig. 3), even though it is known that LPS accelerates monocyte adherence (Doherty et al., 1989). These factors did, however, modify the amount of mRNA encoding the three early response genes (Fig. 7). This mRNA modulation could not be explained by the small increase in the percent of cells which were adherent in the presence of these stimuli (Table I), as this did not affect the adherence-dependent increase in PDGF(B) mRNA. Thus, the present study suggests that PDGF(B) mRNA and mRNA of the three early growth response genes are not coordinately regulated. This does not rule out, however, a complex interaction between the early growth response elements and later PDGF(B) mRNA accumulation.

Role of Early Growth Response Proteins in Differentiation of Monocytes to Macrophages

Fos protein forms a DNA-binding complex with the protein encoded by the jun/AP1 (c-*jun*) gene (Sassone-Corsi et al., 1988*a*,*b*) and may modulate transcription of other genes (Setoyama et al., 1986). A third gene, EGR2, encodes a protein with "zinc finger" structure, a characteristic suggestive of DNA-binding capability (Joseph et al., 1988). Activation of c-*fos* has been implicated in macrophage differentiation as it occurs in human cell lines induced to differentiate to macrophage-like cells (Muller et al., 1984, 1985; Mitchell et al., 1985). In previous studies on the human promyelocytic cell line HL60, we identified specific sequences of mRNA accumulation during differentiation to macrophage-like cells (Shaw, R. J., R. A. F. Clark, V. P. Sukhatme, A. G. Ritter, P. A. Campbell, and S. H. Benedict, manuscript submitted for publication). There were differentiation pathway–specific increases in mRNA encoding c-*fos*, EGR2, c-*jun*, and PDGF(B). However, only during macrophage-like differentiation were all four of these genes activated.

The present study extends these observations and provides the first demonstration of the involvement of c-*jun* and EGR2 in monocyte differentiation to macrophages using freshly isolated, nontransformed cells. We also confirmed the work of others (Haskill et al., 1988) that there is a brief but marked increase in c-*fos* 20 min after monocyte adherence and that activation of the monocyte HLA-DR-α gene is not dependent on adherence or cytoskeletal rearrangement.

The importance of adherence in c-*jun* and EGR2 mRNA accumulation suggests that adherence itself may be the initiating event in the conversion of a circulating monocyte to a tissue macrophage. The inhibition by cytochalasin D of the increases in c-*jun* and EGR2 mRNA further implies that

cytoskeletal integrity may be a crucial additional step before the full pattern of differentiation-associated mRNA accumulation.

There was a divergent pattern of ligand-specific modulation of PDGF(B), c-*jun*, and EGR2 mRNA. Fn caused a modest increase in PDGF(B) mRNA while collagen and LPS had little effect on PDGF(B) mRNA. By contrast, Fn and collagen weakly inhibited the increase in c-*jun*, whereas LPS tended to increase c-*jun* mRNA. Furthermore, all three factors decreased EGR2 mRNA. There was also a difference in the time course of activation of these three genes in that PDGF(B) was maximal at 6 h and decreased by 24 h, whereas c-*jun* and EGR2 were sustained at high levels at 24 h. Thus, these three genes were not coordinately regulated and the increase in PDGF(B) could not be attributed solely to prior activation of c-*jun* and EGR2.

The modulation of c-*jun* and EGR2 by external stimuli as observed during adherence to collagen-coated plates may provide one mechanism by which these stimuli result in differentiation to macrophages with different functional capabilities. For example, monocyte differentiation on collagen-coated plates results in macrophages with enhanced Fc- and C3-mediated phagocytosis (Kaplan and Gaudernack, 1982) and increased TNF-α gene activation (Eierman et al., 1989).

In murine macrophages, there is an inverse relationship between the ligand-specific changes in c-*fos* mRNA and mRNA levels of urokinase-type plasminogen activator (Collart et al., 1987). There are similar associations of patterns of increase in early growth response gene mRNA with activation of later genes associated with functional activities in HL60 cells (Shaw, R. J., R. A. F. Clark, V. P. Sukhatme, A. G. Ritter, P. A. Campbell, and S. H. Benedict, manuscript submitted for publication). The present study, which found an association between early growth response and PDGF(B) mRNA accumulation, and monocyte adherence, lends support to the concept that monocyte adherence may trigger specific sequences of events that lead to divergent macrophage differentiation.

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