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) (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), chemotactants (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.
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; Raghun 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) mRNAs 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 adherent step using a 12:21 centrifuge equipped with a stroke rpm IE-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 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 L-8-80 ultracen trifuge and SW-55 rotor (Beckman Instruments, Inc.) (Glaissin 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 mM formamide, 0.05 M 3-[N-morpholino]propanesulfonic acid (Sigma Chemical Co.), RNA (15 µg/well) was electrophoresed through an agarose/formamide 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 Na2HPO4, 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-32P) dCTP (Amersham Corp., Arlington Heights, IL) (Feinberg and Vogelstein, 1983). Specific activities of probes were ~10⁶ 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-adherance 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-fos fragment (gift of M. Murray, ZymoGenetics, Seattle, WA), a 1.65-kb Eco RI TGF-β fragment (gift of A. Puchito, Oncogen, Seattle, WA), a 1.9-kb Nae I c-fos fragment (cTCC 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 mRNAs, 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-adherance 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
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, 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 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 ≥ 3).

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-

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* 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.
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).
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. 6a). The pooled results of several experiments are shown in Fig. 6b. 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 ≥ 4).
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 ≥ 3).

The 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 (Schiwa, 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-
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/AP-1 (c-jun) gene (Sassone-Corsi et al., 1988a,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 coordinated 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 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 Fn- 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 the 5'-flanking region of the c-fos gene (Kaplan and Gaudernack, 1982). There are similar associations of patterns of increase in early growth response gene mRNA with activation of late genes associated with functional activities in HL60 cells (Shaw, R. J., R. A. F. Clark, V. P. Sukhtatme, 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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References


