Mode of Fibroblast Growth Enhancement by Human Interleukin-1

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Abstract. Previous studies have demonstrated that interleukin-1 (IL-1) stimulates fibroblast growth (Schmidt, J. A., S. B. Mizel, D. Cohn, and I. Green. 1982. *J. Immunol.* 128:2177–2182) and binds to specific, high affinity receptors of BALB/c3T3 cells (Bird, T. A., and J. Saklatval. 1986. *Nature (Lond.).* 324:263–265, 266–268). We have investigated the mechanism of fibroblast growth stimulation by IL-1. Addition of fibroblast growth factor derived from platelets (PDGF) to a quiescent culture of BALB/c3T3 cells produced 8–10-fold increase in DNA synthesis during 24-h incubation. The cellular action of PDGF was mediated through competence induction and required synergistic action of plasma-derived factors for full mitogenic activity. When tested at a wide range of concentrations (0.1–100 pM), natural IL-1 or recombinant IL-1 produced only a maximum of 5–10% of DNA synthesis elicited in response to PDGF or serum. Induction of DNA synthesis required continuous presence of IL-1 and did not exhibit synergism with plasma. Competence induction and mitogenic stimulation by PDGF was associated with early induction of proteins P32, P38, P46–48, P75, and changes in cytoskeletal organization. Examination of these early cellular changes showed that IL-1 did not produce similar induction of cellular proteins and the morphological changes associated with growth stimulation. These results suggest that the mode of IL-1 action on BALB/c3T3 was not through competence induction. When IL-1 was added to cells rendered competent by brief exposure to PDGF, 10–15% additional DNA synthesis occurred during the first 24 h. Extended incubation of PDGF-treated cells in the presence of IL-1 revealed that the stimulation by IL-1 occurred predominantly during the subsequent cycle of DNA replication, wherein DNA synthesis reached three- to fivefold higher than the untreated cultures. We conclude (*a*) IL-1 alone is not a potent mitogen for BALB/c3T3 cells, and does not bring cells out of the growth arrest G_0 phase, (*b*) treatment with PDGF renders the cells more responsive to IL-1, (*c*) part of the IL-1 action on competent cells may be characterized as progression inducing activity, further, (*d*) our results indicate that action of IL-1 on PDGF-treated cells produces sustained DNA synthesis for an extended period, perhaps by preventing the entry of cells into growth arrest G_0 phase.

INTERLEUKIN-1 (IL-1)[1] is known to induce a wide range of biological activities in numerous cell types (21, 23, 31). In connective tissue cells, IL-1 has been shown to stimulate prostaglandin production (15), collagenase production (15, 28), and proliferation of human dermal fibroblasts (29) and BALB/c3T3 cells (12). Recent studies have demonstrated occurrence of high affinity IL-1 receptors on a variety of cell types, including human lung and BALB/3T3 fibroblasts (6, 12, 14). Interestingly, the number of IL-1 receptors expressed by fibroblasts is relatively high as compared with lymphoid cells (6, 9, 13, 14). Although, full biochemical and biological characterization of IL-1 receptor is yet to be achieved, the molecular size of the IL-1 receptor appears much smaller (82 kD) than the other well characterized growth factors, e.g., platelet derived growth factor (PDGF), fibroblast growth factor (FGF), or epidermal growth factor (EGF), whose receptors are 160,000 D or greater (10, 17, 18, 22). It is, therefore, of great interest to know which receptor-mediated signal transduction mechanisms and the early cellular events induced by IL-1 are common to those triggered by other growth factors of BALB/c3T3 cells. Such studies may further advance our understanding of the key steps involved in the regulation of cell proliferation. Previous studies have demonstrated that specific growth factors are functional during defined stages of cell cycle and induce specific biochemical events of the pathway(s) leading to the traverse of quiescent cells from growth arrest G_0 to DNA replicative S phase. Accordingly, initiation of DNA synthesis and proliferative response are brought about by a synergistic action of two types of polypeptides growth factors. One

1. *Abbreviations used in this paper*: EGF, epidermal growth factor; FGF, fibroblast growth factor; IL-1, interleukin-1; PDGF, platelet-derived growth factor.

set of factors, e.g., PDGF, FGF, and thrombin confer a state of competence (competence factors), whereby treated cells come out of the growth arrest G_0 phase and enter the DNA synthesis phase when supplied with the second set of factors known as progression factors (EGF, insulin-like growth factor, plasma) (25, 26, 32). How IL-1 induces fibroblast proliferation is not known. The present study has revealed a novel action of IL-1 on fibroblast. It is shown here that unlike PDGF or FGF, IL-1 does not stimulate growth arrested cells to become competent or enter DNA synthesis phase. Instead, IL-1 acts on competent or exponentially growing cells where it induces their traverse from G_0/G_1 into S phase and sustains growth by preventing entry of cells in growth arrest G_0 phase.

Materials and Methods

Cell Culture

Stock culture of BALB/c3T3 cells was maintained as previously described (25). Growth medium was DME containing 10% FBS, L-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 μg/ml). Assay medium was DME containing indicated concentration of platelet poor plasma, L-glutamine (2 mM), penicillin (100 U/ml), streptomycin (100 ug/ml), and [^{3}H]thymidine (5 μCi/ml).

IL-1

Natural human IL-1 was purified from lipopolysaccharide stimulated peripheral blood leukocyte conditioned medium by sequential chromatography on hydroxylapatite, TSK-3000, and SP-TSK columns (11). Greater than 90% of the IL-1 activity in this preparation was due to IL-1β. Recombinant IL-1α was from Genzyme Corp., Boston, MA. Recombinant IL-1β was prepared at the Upjohn Company or purchased from Genzyme Corp. FGF was from Collaborative Research, Inc., Bedford, MA.

PDGF

Human PDGF was purified by modification of previously described method (4, 30). Platelet extract fractionated on CM-Biogel was further purified on molecular sizing TSK-3000 and cation exchange SP-TSK column using HPLC. The specific activity of purified PDGF was 0.5 × 10^6 U/mg protein. A unit of PDGF is defined as the amount that induces 50% of maximum DNA synthesis.

Platelet Poor Plasma

Human platelet poor plasma and plasma from hypophysectomized rats were prepared as before (25).

Competence and Progression Assays

BALB/c3T3 cells were cultured in 0.2 ml of growth medium at a density of 4 × 10^3 cells per well of a 96-well microtiter plate. Plates were incubated at 37°C under 5% CO_2, 95% air, and used for assay after 5 d. For competence assay, cells were washed in DME and medium was replaced with assay medium containing 1-2% human platelet poor plasma, and appropriate growth factor. Plates were incubated for 24 h as before. At the end of incubation, cells were fixed in methanol. The microwells were detached from the plates and radioactive thymidine incorporation was determined by scintillation counting using a Beckman counter (model No. LS3801; Beckman Instruments, Inc., Palo Alto, CA). For progression assay, cells were first treated with 1-2 U PDGF for 4 h, washed, and either transferred to assay medium containing low concentration (0.5%) of human platelet poor plasma or 2% hypophysectomized rat plasma and various concentrations of IL-1. [^{3}H]Thymidine was added at the start for a 24-h assay and after 24 h for a 48-h assay.

Two-dimensional Gel Electrophoresis

Two-dimensional gel electrophoresis of proteins was performed by a modification of the methods described by Anderson and Anderson (2, 3). First-dimension tube gels were run using pH 3.5 to 10.0 ampholytes. The second dimension, SDS-PAGE was performed using 10–20% gradient gels (1).

Results

Fig. 1 shows the effects of two classes of growth regulators on DNA synthesis in BALB/c3T3 cells. Addition of competence factor PDGF to a quiescent culture of BALB/c3T3 cells produced a dose-dependent increase in DNA synthesis as determined in a 24-h competence assay. Growth stimulation by PDGF was synergistically enhanced by plasma. In the absence or low concentration of plasma (0.1%, in Fig. 1), 120 pM PDGF was required to achieve one-half of maximum DNA synthesis. Increasing the plasma concentration to 1% produced greater than two-fold enhancement of PDGF activity (50 pM PDGF was needed to produce half-maximum DNA synthesis). Higher concentrations of plasma were even more effective; however, the background radioactivity was also increased. To keep the background radioactivity low, most of our experiments used 1–2% plasma. It should be noted that the data in Fig. 1 are plotted after subtraction of background for each plasma concentration and the [^{3}H]thymidine uptake shown here represents stimulation by growth factors. These results are consistent with previously described mode of BALB/c3T3 cell proliferation in response to growth factors (25, 26, 32). Fig. 1 and at an expanded scale in the inset of Fig. 1 show stimulation of DNA synthesis in BALB/3T3 cells in response to recombinant IL-1β. Similar results were obtained with IL-1α or IL-1 purified from lipopolysaccharide stimulated PBL-conditioned medium. IL-1 at concentrations between 0.2 and 2 pM produced a slight in-

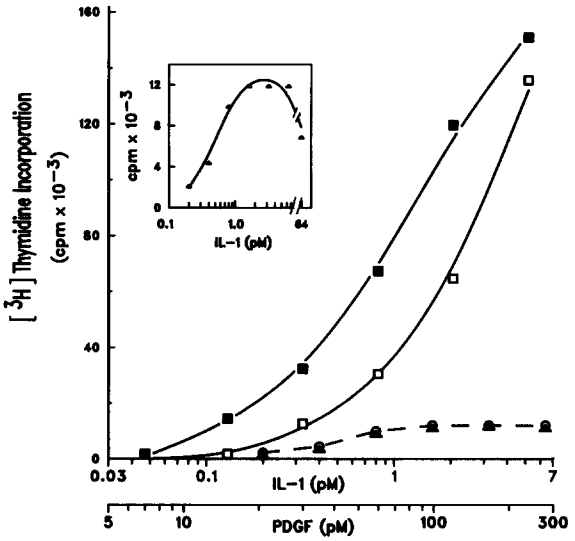

Figure 1. Induction of DNA synthesis in BALB/c3T3 fibroblasts by human IL-1 or PDGF. BALB/c3T3 cells were plated in 96-well microtiter plates and allowed to become confluent in DME containing 10% FBS. 5 d after plating, spent medium was replaced with DME containing appropriate concentration of platelet poor plasma, indicated amounts of PDGF, or IL-1, and 1 μCi [^{3}H]thymidine (sp act 78 μCi/mmol). Results of [^{3}H]thymidine incorporation in response to PDGF in 1 (■) or 0.1% (□) platelet poor plasma, IL-1 in 1 (▲) or 0.1% (○) platelet poor plasma are plotted after subtraction of background radioactivity for each plasma concentration. For clarity of depiction DNA synthesis in response to IL-1 is plotted at a 10-fold expanded scale in inset.

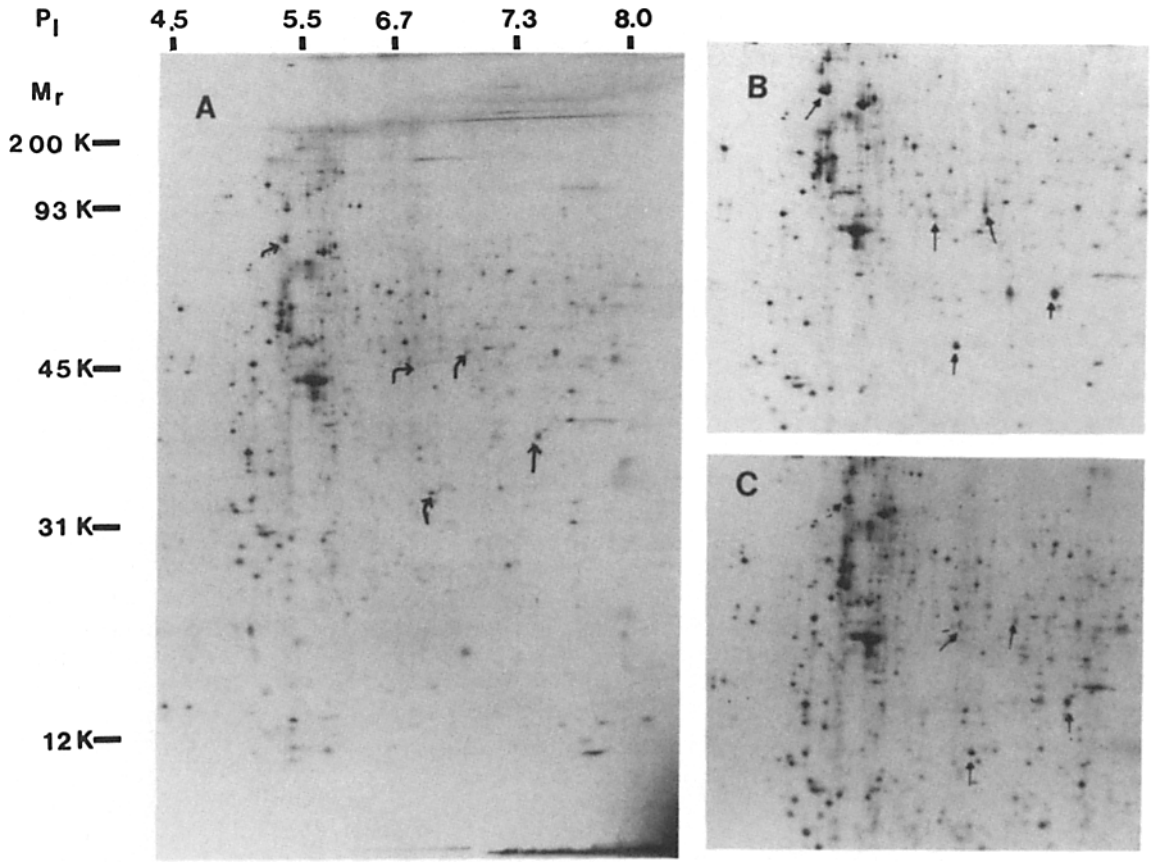

Figure 2. Two-dimensional gel electrophoresis of PDGF, IL-1, or platelet poor plasma–treated control cells. BALB/c3T3 cells were grown to confluence in 35-mm dishes and then transferred to 2 ml DME containing 1% plasma (*A*), and 250 pM PDGF (*B*), or 10 pM IL-1 (*C*) for 2 h before a 30-min pulse with 50 μCi [^{35}S]methionine. Cultures were washed and cells were lysed in a 1% solution of NP-40 in reticulocyte saline buffer (0.01 M NaCl/0.01 M Tris-HCl/1.5 mM $MgCl_2$ pH 7.4) and supernatants were subjected to first dimension electrophoresis between pH 3.5 and 10 and second dimension using 10–20% gradient polyacrylamide gels. Autoradiographs from a representative experiment are shown here. Proteins consistently induced two- to fourfold and quantified in Table I are marked by arrows.

crease in DNA synthesis by fibroblasts. However, the thymidine incorporation in response to optimum concentration of IL-1 was only 5–10% of that produced by PDGF or serum. In various experiments performed with different batches of cells or different preparations of IL-1, the maximum thymidine incorporation in IL-1–treated cultures was 8,000–12,000 cpm or less (3,000–6,000 cpm) if the growth of cells was further arrested by incubation in the growth factor–depleted medium for 6-d instead of 5-d cells routinely used for the assay. In PDGF-treated cultures the maximum radioactivity incorporated in DNA was 150,000–200,000 cpm. Unlike PDGF, IL-1 activity was not dependent on the concentration of platelet-poor plasma in the medium. Fig. 1 shows the [^{3}H]thymidine incorporation in response to IL-1 in the presence of 0.1 and 1% platelet poor plasma. In other experiments, not shown here, varying the plasma concentration between 0.1 and 3% did not significantly change the IL-1 response. The extent of stimulation of DNA synthesis by IL-1 found here was contrary to the previous suggestions that IL-1 is a growth factor for fibroblasts. Lack of induction of DNA synthesis by IL-1 under our experimental conditions was not due to the inactivation of IL-1 by human plasma. IL-1 diluted in human platelet poor plasma was equally active in thymocyte assay. Lack of growth stimulation was also not due to inhibition by prostaglandin produced in response to IL-1 or any nonspecific inhibition by IL-1 preparation, because IL-1–treated cells were fully responsive to other mitogens of BALB/c3T3 cells (see below, Fig. 4). These results suggest that IL-1 does not stimulate DNA synthesis in growth-arrested cultures. A 5–10% increase in DNA synthesis observed in the presence of IL-1 is perhaps due to its action on a small population of nonsynchronized cells still cycling. These experiments suggested that the mode of fibroblast growth stimulation by IL-1 (as reported before), may be different from the previously described fibroblast growth factors e.g., PDGF or FGF.

Further evidence that IL-1–mediated proliferative response was different from the other growth factors is derived from examination of early biochemical events associated with the action of PDGF or FGF. Fig. 2 shows that treatment of density-inhibited BALB/c3T3 cells with PDGF induced increased expression of specific cellular proteins within 1–2 h, as determined by [^{35}S]methionine labeling of cellular proteins. A relative fold induction of specific proteins corresponding to M_r 32,000, 38,000, 46,000, 48,000, and 75,000 was determined by scanning the autoradiograms. The relative integrated intensity of the protein of interest in each gel was compared with a reference protein (protein exhibiting

Table 1. Induction of Cellular Proteins by PDGF and IL-1

Protein	Molecular mass	pI	Optical density: Platelet poor plasma	Optical density: PDGF	Optical density: IL-1
	$\times 10^{-3}$				
P32	32	6.8	0.80	3.64	1.48
P38	38	7.3	1.40	3.24	1.86
P46	46	6.7	0.49	2.18	0.50
P48	48	7.0	0.29	1.21	0.38
P75	75	5.2	1.55	3.82	2.28

Individual proteins in autoradiographs were first identified visually and then quantified by computer aided densitometry using a Visage image analysis system (Biolmage Corp., Ann Arbor, MI). Optical densities (in arbitrary units) were determined by dividing the integrated intensity of each protein shown by the integrated intensity of a reference protein that exhibited none or smallest change in response to growth factor treatment. Data presented here are average of two separate experiments.

none or smallest change) and expressed in arbitrary units. Table I shows that treatment with PDGF produced four- to fivefold induction of P32, P46, P48, and two- to threefold increase in the synthesis of P38 and P75. Similar increase in synthesis of 29- and 35-kD proteins was reported by Pledger et al. (33). The 29-kD protein corresponds to our P32. Although our results show a 2–3-kD higher molecular mass for this protein, more recent experiments from the laboratory of the investigators confirms this result (Pledger, W. J., personal communication). Similarly, the 35-kD protein may correspond to our P38. In this study we also found that the 46- and 48-kD proteins, expressed at a very low level in growth-arrested cells, are stimulated severalfold upon PDGF treatment. IL-1, tested at several concentrations, did not produce significant change in the expression of P38, P46, and P48, and only a slight increase in P32. At higher concentrations (20 pM), IL-1 may have actually inhibited the expression of these proteins (unpublished observations).

It is known that cell shape changes are important determinants in the regulation of cell growth, migration and differentiation. DNA synthesis and mitosis in fibroblast treated with PDGF are preceded by changes in actomyosin cytoskeletal system, resulting in changes in cell shape. Fig. 3 shows the effect of PDGF on morphology of BALB/c3T3 cells. After reaching a near confluent density, cells in control cultures remained uniformly hexagonally or pentagonally shaped with flattened morphology, covering much of the dish surface. PDGF treatment produced a striking change in morphology and cell shape characterized by cell elongation. Induction of cell stretching was a function of PDGF concentration and enhanced by plasma. IL-1 tested at several concentrations (0.1–10 pM) or extraphysiological concentration (100 pM) did not produce any visible change in morphology of these cells. Taken together, the DNA synthesis experiments and the examination of other cellular changes associated with growth factor–induced cell proliferation, we conclude that IL-1 alone is not a potent mitogen or competence-inducing factor for fibroblasts.

The second set of factors required for the traverse of BALB/c3T3 cells from G_0/G_1 to S phase of cell cycle are present in plasma (progression factors). Much of the progression activity of human plasma is due to somatomedin C, the plasma concentration of which is under the control of pituitary hormone. We have used somatomedin C–deficient plasma from hypophysectomized rats or low concentration of human plasma to determine whether the induction of DNA synthesis in BALB/c3T3 cells by IL-1 was due to its action as a progression factor. In Fig. 4 *A*, cultures of BALB/c3T3 cells were rendered competent by brief exposure (4 h) to human platelet-derived growth factor at a concentration of 1–2 U/ml. As indicated by the open bar in Fig. 4 *A*, PDGF in the presence of optimum concentration of human platelet-poor plasma produced about sixfold increase in DNA synthesis (124,200 ± 1,400 cpm above background). In progression factor–deficient hypophysectomized rat plasma or low concentration of human platelet-poor plasma, a 4-h exposure to PDGF produced ~30–40% (41,700 ± 150 cpm) of this maximum DNA synthesis. Addition of IL-1 to PDGF-

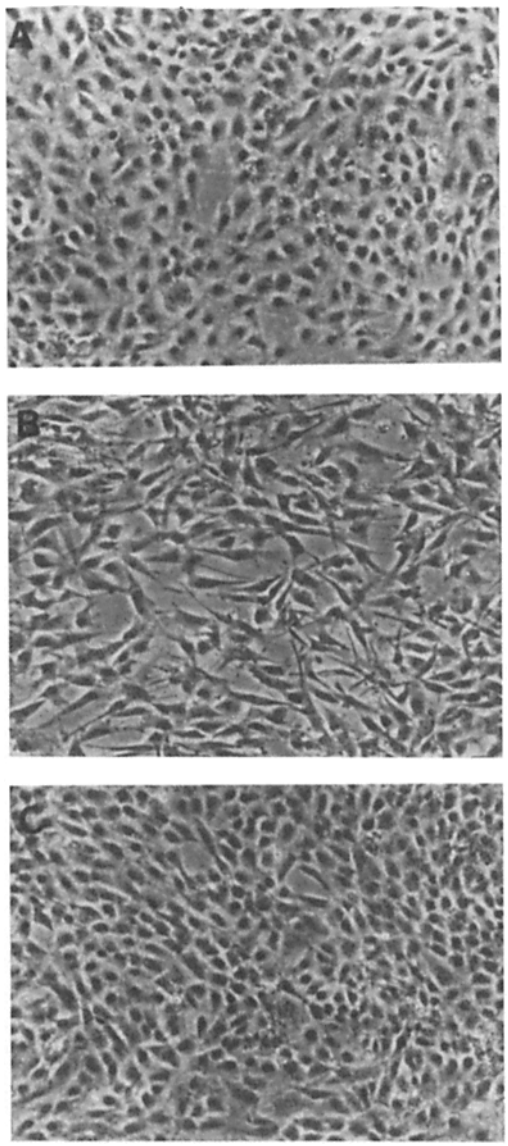

Figure 3. Morphological changes associated with mitogenic stimulation of BALB/c3T3 cells with PDGF. Cells were grown to confluence and then transferred to 1% plasma (*A*) and treated with 250 pM PDGF (*B*) or 10 pM IL-1 (*C*). After 24 h, cells were fixed in methanol and photographed.

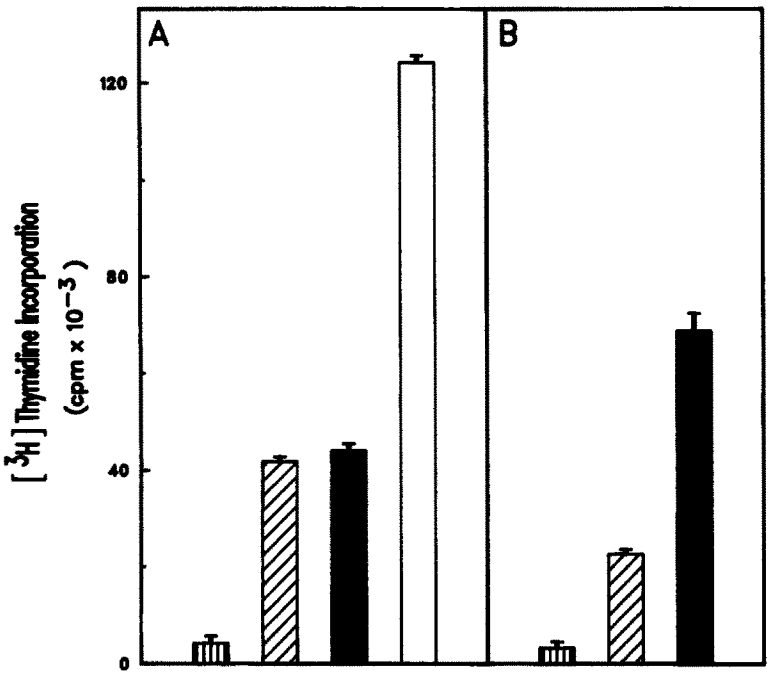

Figure 4. Stimulation of DNA synthesis in PDGF-treated cells by IL-1. Density arrested BALB/c3T3 cells in 96 well microliter plates were washed once with DME and then treated with PDGF (1 U/ml) for 4 h. Control cultures received PBS instead of PDGF. After 4 h cells were washed and transferred to culture medium containing 0.5% platelet poor plasma as follows: control cultures + 2 pM IL-1 (▥); PDGF-treated cultures (▨); PDGF-treated cultures + 2 pM IL-1 (■). The open bar (□) shows [^{3}H]thymidine incorporation in cultures optimally stimulated with PDGF and 2% platelet poor plasma. The later also indicates that the cells were fully capable of undergoing DNA synthesis when supplied with optimum concentrations of PDGF and plasma factors. In *A*, [^{3}H]thymidine was added at the start of the incubation after PDGF pulse and DNA synthesis determined during the first 24 h. In *B*, [^{3}H]thymidine was added after 24 h and DNA synthesis determined during 24–48 h interval. The data are plotted after subtraction of background counts obtained in the absence of treatment as SD of mean, $n = 3$.

treated competent cells produced a slightly higher level of DNA synthesis. As shown in Fig. 4 *A*, this increase in DNA synthesis during the first 24 h was ~2–5% more than the additive effects of PDGF and IL-1. These results suggest that IL-1 may enhance DNA synthesis in competent cells but unlike insulin-like growth factor or FGF it does not fully restore the progression activity of plasma. When the cells first treated with PDGF for 4 h, and then incubated with IL-1 and DNA synthesis determined during 24–48-h interval, IL-1–induced DNA synthesis was greatly enhanced. Fig. 4 *B* shows the DNA synthesis in response to optimum concentration of IL-1 in the presence or absence of PDGF pretreatment. In the cultures receiving IL-1 alone, DNA synthesis during 24–48-h interval did not change significantly, as compared with 0–24-h interval. DNA synthesis in cultures that were pulsed with PDGF declined to ~50% of the initial stimulation (from ~41,700 ± 150 cpm during 0–24 h to 22,500 ± 280 cpm). During the same interval, DNA synthesis in PDGF-treated cultures receiving IL-1 was 166% higher than the additive effect of PDGF and IL-1. Further, during the 24–48-h interval, the IL-1–mediated DNA synthesis in PDGF-treated cells was ~11-fold higher than cells treated with IL-1 alone.

Table II. IL-1–mediated DNA Synthesis in BALB/c3T3 Fibroblasts

Treatment	Labeled nuclei/field	
	0–24 h	24–48 h
Platelet poor plasma	17.5 ± 2.0	11.0 ± 1.0
IL-1	17.0 ± 1.1	7.0 ± 1.0
PDGF	63.2 ± 7.2	17.0 ± 1.0
PDGF + IL-1	66.5 ± 5.8	41.5 ± 2.0

BALB/c3T3 cells were plated as described previously in legend to Fig. 1. Spent medium from the confluent cultures was replaced with DME containing 0.5% platelet poor plasma and 2 pM recombinant IL-1 β or 1 U/ml PDGF or both PDGF and IL-1. Cells were pulsed with [^{3}H]thymidine during 0–24 or 24–48 h interval. At the end of the incubation, cells were fixed in methanol. Autoradiography was performed as described before (4). Labeled nuclei were counted under 10× magnification. Each treatment was performed in triplicates. Data indicates the standard error of mean.

A similar pattern of stimulation of DNA synthesis by PDGF and IL-1 was revealed when the activities were determined by assessing the labeled nuclei after autoradiography. As shown in Table II, suboptimum concentration of PDGF (1 U/ml) produced about four- to fivefold increase in number of labeled cells in a confluent culture. Addition of IL-1 did not significantly change the number of labeled nuclei during the first 24 h. However, during the 24–48-h [^{3}H]thymidine pulse, the number of labeled nuclei in cultures treated with PDGF and IL-1 together was about fourfold higher than PDGF alone (calculated after the subtraction of background labeling in the presence of platelet poor plasma). These results also show that only ~17% of the cells that were initially (0–24 h) stimulated by PDGF, actively synthesized DNA during the 24–48-h interval; whereas, ~64% of the cells actively synthesized DNA in cultures treated with both PDGF and IL-1 during the same interval. One interpretation of this sustained DNA synthesis may be that the action of IL-1 allowed reentry into S phase of cells that were initially stimulated with PDGF. In the absence of IL-1 these cells would have entered G_0 phase and become growth arrested. IL-1 was an extremely potent mediator of augmentation of DNA synthesis in PDGF-treated cells. Maximum effects on BALB/c3T3 were produced with as low as 2 pM IL-1. These levels of IL-1 were 5–10-fold lower than required for stimulation of mouse thymocytes.

We also examined whether the observed effects of IL-1 on DNA synthesis in confluent cultures are reflected in the actual increase in cell number in sparse cultures. In the experiment shown in Fig. 5, cell proliferation over a 4-d period was determined in the presence of suboptimum concentration of PDGF (1 U/ml), 2 pM IL-1, or PDGF and IL-1 together. The change in cell number over the control cultures (2% platelet poor plasma) are shown here. Clearly, addition of IL-1 to the PDGF-containing cultures resulted in higher cell growth.

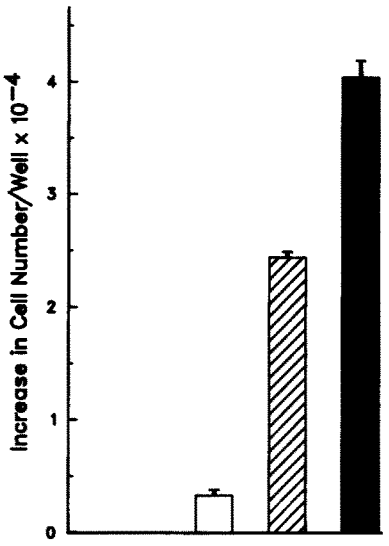

Figure 5. Proliferation of BALB/c3T3 fibroblasts in response to PDGF and IL-1. Stock cultures were trypsinized and cells were plated in 12 well culture plates, and 2 ml DME containing 2 mM glutamine, penicillin (100 U/ml), streptomycin (100 μg/ml), and 2% platelet poor plasma. After 24 h, medium and floating cells were removed and replaced with the above fresh medium containing either 2 pM IL-1 (□), or suboptimal concentration (1 U/ml) of PDGF (▨), or PDGF and IL-1 together (■). Plates were incubated at 37°C in 5% Co_2, 95% air. At the end of incubation, cultures were trypsinized and counted using a Coulter counter (model ZM; Hialeah, FL). The data indicate increase in cell number after 4 d, over the platelet poor plasma control culture (0.4×10^4 cells/dish).

Discussion

Conflicting reports have appeared on growth promoting effects of IL-1 on mesenchymal cells (7, 11, 12, 27, 29, 33). Schmidt et al. (29) initially demonstrated that the fibroblast growth-stimulating activity of the mixed lymphocyte supernatant was biochemically identical to IL-1, and suggested that IL-1 may function as a fibroblast growth factor. Similar proliferation of human dermal fibroblasts in response to IL-1 was subsequently reported by Kimball et al. (19). More recently Dower et al. (14) and Bird and Saklatvela (6) showed that BALB/c3T3 cells exhibit high affinity cell surface receptors for IL-1. Dower et al. (12) also showed a small enhancement of DNA synthesis in BALB/c3T3 cells by IL-1. However, other reports show that IL-1 alone was ineffective in stimulation of growth of fibroblasts (7, 11, 27, 33) and smooth muscle cells (20).

When the fibroblast-stimulating activity of IL-1 was examined in an assay similar to that widely used for the determination of PDGF or FGF, we found that IL-1 produced only a small change in DNA synthesis. Compared with the DNA synthesis induced by PDGF or serum, in most cases, IL-1 stimulation was ~5%. These results were contrary to the previous suggestion that IL-1 is a growth factor for fibroblasts. Detailed investigation of IL-1 activity on G_0 growth-arrested, PDGF-treated competent cells and cycling cells have indicated a novel action of IL-1 on fibroblasts. It is demonstrated here that IL-1 alone does not release G_0-arrested, quiescent fibroblasts to enter DNA replicative S phase. The DNA synthesis results are corroborated by studies showing that IL-1 failed to induce specific cellular changes associated with mitogenic stimulation of BALB/c3T3 cells with PDGF. PDGF treatment of quiescent cultures of BALB/c3T3 cells produced a three- to fivefold increase in the expression of several proteins. Expression of these proteins is coupled with mitogenic stimulation of these cells. Transformed variants of BALB/c3T3 cells, which do not require exogenous PDGF for growth, express the PDGF-inducible proteins constitutively (24). In this regard, our quantitation of PDGF-inducible proteins reveal that proteins P46 and P48, which are produced at a very low level in growth-arrested cells, are strongly induced by PDGF. The induction of these proteins may have remained undetected in previous studies. Stimulation of BALB/c3T3 cells with PDGF or serum, occurs with a concomitant alteration in cytoskeletal organization resulting in cell shape changes. Changes in cell shape are important for cell growth (5, 8, 16). IL-1 added to growth-arrested cells neither produced a significant increase in the expression of the above cellular proteins nor the morphological changes. These results clearly showed that IL-1 was not effective in releasing the cells from the growth arrest G_0 phase.

We show that action of IL-1 is on competent and exponentially growing cells, prestimulated by mitogens such as PDGF. On competent cells, part of the DNA synthesis in response to IL-1 may be characterized as progression inducing activity, allowing the traverse of cells through G_0/G_1 into S phase. However, the IL-1 activity on BALB/c3T3 cells appears different from the previously characterized progression factor EGF or insulin-like growth factor (25, 26, 32). First, EGF alone stimulates DNA synthesis in BALB/c3T3 cells, which is two- to threefold more than IL-1 alone (data not shown), although, EGF-stimulated DNA synthesis was only ~30% of that stimulated by PDGF. Second, the synergism between PDGF and EGF can be observed in a 24-h competence/progression assay (25, 26, 32). As shown here (Fig. 4 and Table II), the stimulation of DNA synthesis by IL-1 occurred predominantly during a 24–48-h interval. Brief exposure of quiescent cells to a suboptimum concentration of PDGF stimulated DNA synthesis during the first 24 h. Unless PDGF treatment was repeated, DNA synthesis declines and cells become growth arrested. When IL-1 was present in the culture medium, DNA synthesis was maintained about three- to fourfold greater than PDGF alone or 11-fold higher than IL-1 alone (Fig. 4 *B*). The enhanced DNA synthesis may result from reentry into S phase of cells emerging from the first round of DNA replication initiated by PDGF. This may occur if IL-1 somehow prevents the entry of cells into growth arrest G_0 phase. These data also explain the IL-1 induced fibroblast growth stimulation reported by Schmidt et al. (29) and Kimball et al. (19). Their studies used serum (instead of plasma), which contained 1–2 ng PDGF and plated cells at low densities. Under these conditions a larger population of cells are expected to remain nonsynchronized, out of G_0 arrest.

The data presented here demonstrate the role of IL-1 in the regulation of fibroblast growth through a unique mechanism. These findings reveal an interesting system to study a very poorly understood phenomenon of G_0 growth arrest. These studies also suggest that a collective action of the two macrophage derived monokines, IL-1 and a macrophage-derived growth factor may constitute a complete system for prolifera-

tive responses in wound healing, inflammation, and atherosclerosis where large number of macrophages are found deposited.

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