Contractile Proteins Participate in Release of Erythroid Growth Regulators From Mononuclear Cells

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We have investigated the role of contractile proteins of circulating mononuclear cells in generation of membrane-associated, erythroid growth regulatory molecules. Lymphocytes and monocytes were incubated under serum-free conditions without and with cytochalasin B, cytochalasin D, or colchicine, and effects on positive and negative erythropoietic activities were determined in cell membranes and in surface membrane vesicle-rich pellets and supernatants of dialyzed medium conditioned by the cells. In serum-free cultures of human bone marrow, plasma membranes and exfoliated membrane-derived vesicles from cytochalasin-treated lymphocytes lost their capacity to support the formation of erythroid bursts, while monocyte membrane-associated inhibitory activity was abolished by preincubation with cytochalasin. In contrast, membrane-associated activities of colchicine-treated cells were unaffected. Cytochalasin-induced alterations of membrane regulatory molecules were observed in a dose-dependent fashion over a wide range of concentrations (1 to 100 μg/mL) tested.

However, the capacity of membrane vesicle-free supernatants of medium conditioned by lymphocytes or monocytes was unaffected by cytochalasins, regardless of drug concentration used. Lysates of cytochalasin B-treated cells inhibited the activity of deoxyribonuclease I to a greater degree than did lysates of untreated cells, suggesting that the relative amount of monomeric actin is increased in the cytoplasm of treated cells. Furthermore, results of experiments with D-glucose and with cytochalasin D suggest that cytochalasin effects are independent of alterations in glucose metabolism. The data indicate that expression of plasma membrane-associated regulators is sensitive to agents that block polymerization of actin. They raise the possibility that changes in distribution of actin between unpolymerized and filamentous pools may influence the organization and/or function of mononuclear cell surface-associated erythroid regulatory molecules.

MATERIALS AND METHODS

Exposure of mononuclear cell populations to cytochalasins or colchicine. Peripheral blood and bone marrow cells were collected in alpha-medium (Gibco 325-2561; GIBCO, Grand Island, NY) plus 20 units preservative-free heparin per milliliter from healthy, paid volunteers with prior informed consent and approval of an Institutional Review Board. Light-density mononuclear cells appearing at the interface of medium and Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) were removed with a Pasteur pipette. Circulating mononuclear cells were separated into populations of >95% lymphocytes and >90% monocytes by mixture with carbonyl iron or by adherence to polystyrene, as described previously. Each cell population was split into equal aliquots and suspended to a density of 5 × 10⁶/mL in serum-free alpha-medium with or without cytochalasins or colchicine. Stock solutions of transport mechanisms. Since the regulatory activities in both plasma membranes and shed vesicles from cytochalasin-treated cells are similarly diminished, our results raise the possibility that microfilaments play a role in growth regulator insertion into the plasma membrane.

LITTLE INFORMATION is available concerning how mononuclear cells release hematopoietic growth factors. In addition to exocytosing active factors, mammalian cells may exfoliate membrane-associated regulatory molecules from their surface. The latter process is believed to involve selective pinching off of plasma membrane vesicles at the base of protrusions from the cell surface. Recently, we purified an erythroid burst enhancing molecule from freshly prepared, human lymphocyte plasma membranes and from spontaneously shed, surface membrane-derived vesicles. The active molecule is an integral membrane protein of molecular weight (mol wt) 28,000 on gel filtration that may be important to local communication among hematopoietic cells.

Considerable evidence has accumulated suggesting that intracellular structural proteins may be important to physical as well as functional integrity of cell surface components. For example, exposure of various mononuclear cell types to agents known to rearrange microfilaments or microtubules results in interference with several cell surface-associated processes, including immunoglobulin shedding, hormone-induced membrane ruffling, membrane protein segregation, and leukemic myeloid differentiation. Furthermore, direct assessment of structural proteins has implicated the contractile apparatus in maintaining the physiologic configuration of major histocompatibility complex (MHC) molecules and antigen for immunogenenicity.

To investigate the role of intracellular structural proteins in expression of erythroid growth regulatory molecules, we assayed soluble and membrane-associated activities released from mononuclear cells exposed to cytochalasins or colchicine, disrupters of microfilament and microtubule integrity, respectively. We have found that membrane-associated, stimulatory activity in lymphocytes and inhibitory activity in monocytes are abolished in a concentration-dependent manner by incubation with cytochalasins but not with colchicine. The cytochalasin effect appears to be independent of glucose

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cytochalasin B and cytochalasin D (Sigma Chemical Co, St Louis) were prepared in phosphate buffered saline (PBS) containing 10% dimethylsulfoxide (DMSO), and were added to alpha-medium to obtain final concentrations of 1 to 100 µg/mL (representing molar concentrations of 2.10 to 210 µmol/L cytochalasin B and 1.97 to 197 µmol/L cytochalasin D). In some cases, 0.5 to 1.0 mol/L D-glucose was added to cytochalasin B-containing medium. A stock solution of colchicine (Sigma) was prepared in sterile tissue culture water (Difco, Detroit) and added to alpha-medium to obtain final concentrations of 1 to 100 µg/mL (representing molar concentrations of 2.5 to 250 µmol/L). Cells were incubated in 25-cm² tissue culture flasks (Corning Glass Works, Corning, NY) for 24 hours at 37°C in humidified air, and pelleted by centrifugation at 280 g for 20 minutes. Cell-free conditioned medium (CM) was dialyzed against PBS for seven days, 4°C, with changes three times per day, and passed through a 0.45 µm filter (Millipore Corp. Bedford, MA) before addition to culture. Cell viability was assessed by trypan blue exclusion and by staining with ethidium bromide and acridine orange.

Cell-free whole CM was separated into supernatants and plasma membrane-derived vesicle-rich pellets by centrifugation at 40,000 g for 30 minutes. We found that approximately 50% of stimulatory activity in CM is associated with shed membrane-derived vesicles.1 Translucent pellets were washed three times in 5 mmol/L Na-phosphate, pH 7.6, resuspended in alpha medium and added to culture so that their final concentrations (vol/vol) were equal to that in starting CM, or at the final protein concentrations indicated in Figs 1-7. In some cases, vesicles shed from untreated cells were incubated with 100 µg cytochalasin B per milliliter of alpha medium (24 hours, 37°C), pelleted, washed, and added to culture as described. We have previously shown that under these conditions of reincubation, burst-promoting activity (BPA) remains associated with membrane vesicles in the CM pellet.2 Protein concentrations were estimated by the method of Lowry et al.13 We have previously reported that electrophoresis of CM pellets on 5% sodium dodecyl sulfate (SDS) gels yields a prominent vesicle band at 42,000 daltons after staining with Coomassie blue, which is coincident with the position at which purified actin migrates on similar gels.2 In an experiment in which gels of CM pellets were electroblotted to nitrocellulose paper and then probed with antibodies, immunologic reactivity with anti-actin IgG (ICN Biochemicals, Cleveland) was observed.

Assay for monomeric and filamentous actin. To determine whether the ratio of monomeric actin to filamentous actin is altered following exposure to cytochalasins, extracts of circulating mononuclear cells incubated at 37°C overnight with and without 10 µg cytochalasin B per milliliter, were prepared according to the method of Blikstad et al.15 Briefly, cells were harvested, washed twice in cold PBS, and lysed in 5 mmol/L K-phosphate, pH 7.6, 150 mmol/L Na chloride, 2 mmol/L Mg chloride, 0.2 mmol/L adenosine triphosphate (ATP) (Sigma), dithioerythritol, and 0.5% Triton X-100 plus 0.01 mmol/L phenyl-methyl sulfonyl fluoride (Sigma). Lysates were immediately placed on ice and assayed for monomeric and filamentous actin by inhibition of the enzyme deoxyribonuclease I (DNAse I). DNAse inhibitor activity in the extracts was measured within 60 minutes using calf thymus DNA (Sigma). Between 50 and 150 µL DNAse I (1 to 2 mg/mL; Sigma) was added to 1 mL of DNA (40 µg/mL). For assays of cell extract effects on DNAse I hydrolysis, lysates were added to DNAse I and immediately transferred to DNA solution. In the case where filamentous actin was depolymerized to monomeric actin in order to determine total actin content, an equal volume of depolymerizing solution (guanidine HCl) was added to lysate and incubated on ice for more than five minutes. Fresh ATP was added to the depolymerizing solution before each experiment. Depolymerized extracts were added to enzyme, mixed rapidly, and transferred to DNA solution. Hydrolysis of DNA was quantified by measuring the hyperchromicity at 260 nm using a Perkin-Elmer Dual Beam Lambda 380UV-VIS spectrophotometer. The percentage of actin pools was calculated by determining the ratios of the difference in mean absorbance of (control; five samples) – (test lysate; three samples) polymerized actin divided by the difference in mean absorbance of (control; five samples) – (test lysate; three samples) depolymerized (total) actin. This assay has been reported to detect 10 µg of actin per milliliter.13

Serum-free assay of regulatory activities. Light-density marrow cells were either cultured directly or further separated by adherence to polystyrene, 90 minutes, 37°C; followed by indirect panning with mouse anti-human B4 antibody (100 µg/mL, 30 minutes, 4°C), as described previously.15 Marrow cells were cultured at a density of 6 × 10³/mL under serum-free conditions. Cultures contained Iscove's modified Dulbecco's medium (GIBCO), highly purified human serum albumin, saturated ferric chloride/transferrin, and to 4 U/mL sheep step III erythropoietin (Connaught Laboratories, Ontario) or recombinant human erythropoietin (70,000 to 80,000 U/mg; Amgen Biologicals, Thousand Oaks, CA). Test plates contained 4.5, 9.0, or 18% (vol/vol) CM or CM fractions. In some cases, 9% (vol/vol) cytochalasin B or colchicine was added directly to culture at concentrations indicated in Fig 1. Control plates contained an equal volume of IMDM. Fibrin clots were formed with highly purified fibrinogen and thrombin. Cultures were maintained at 37°C, 5% CO₂ in humidified air. Bursts (or BFU-E derived colonies) consisting of ≥50 nucleated, benzidine-positive cells were scored after 12 days of incubation. Colonies consisting of eight to 49 nucleated, benzidine-positive cells were scored as colony forming unit-erythroid (CFU-E) derived colonies after seven days of incubation. Cloning efficiency varied between 20 and 50 burst forming unit (BFU-E) derived colonies and 25 to 75 CFU-E derived colonies/6 × 10⁶ cells plated.

Extraction of growth factors from shed vesicles and plasma membranes. Purified plasma membranes and membrane vesicle-rich lymphocyte conditioned media (LCM) pellets from both normal and cytochalasin B-treated lymphocytes were extracted with the nonionic detergent octyl β-D-glucopyranoside as previously described.3 The solubilized proteins were dialyzed free of detergent, sterilized by passage through a 0.45 µm filter (Millipore Corp,
Bedford, MA) and assayed in serum-free fibrin clots for stimulatory activity.

Statistical analysis. The number of erythroid colonies formed was determined in each of four 125 μL clots. Means ± SEM of quadruplicate determinations for each test point were calculated and data sets were compared by the two-sample ranks test of Wilcoxon and White.17

RESULTS

Cytoskeletal protein disruptors impair erythroid progenitor proliferation. As shown in Fig 1, proliferation of BFU-E and CFU-E progenitors was abolished by addition of ≥5 μg cytochalasin B directly to bone marrow culture. The degree of inhibition of erythroid colony formation was a function of cytochalasin B concentration with 50% inhibition observed at a concentration of 0.5 μg/mL. Suppression of erythroid colony formation was also observed in cultures of narrow mononuclear cells depleted of accessory lymphocytes and monocytes, although the dose-response curve was shifted to the right (data not shown). Since cytoplasmic actin filaments are actively involved in generating mechanical forces during cell division,18 inhibition by cytochalasin B may be due to impairment of bone marrow cytokinesis per se. On the other hand, cytochalasin B effects may also be a result of inhibition of endogenous release of regulatory factors from neighboring marrow cells in vitro.

Cytochalasins alter release of erythroid regulators. To separate direct marrow progenitor cell effects from those exerted via accessory hematopoietic cells, we prepared LCM media conditioned by monocytes (MCM) in the absence and presence of cytochalasin B. We have previously shown that LCM contains a pelletable stimulatory factor for BFU-E proliferation, while MCM contains a pelletable vesicular factor that inhibits proliferation of these cells.19 In some cases, cytochalasin B was removed from the media by dialysis before addition of CM to marrow culture. Figure 2A shows that dialysis had no effect on positive or negative regulator expression in whole media prepared from untreated cells. In contrast, the inhibitory effect of cytochalasin B diluted in culture medium (without added cells) was removed by dialysis (Fig 2B). Undialyzed LCM and MCM prepared in the presence of 20 μg cytochalasin B per milliliter showed no stimulatory activity and little inhibitory activity (at 18% vol/vol; P > .05 relative to growth without added MCM), respectively (Fig 2). Dialysis only partially restored the capacity of LCM prepared from cytochalasin B-treated cells to support erythroid burst formation (Fig 2C), while it fully removed inhibitory activity from MCM (Fig 2D). The data are consistent with the possibility that the release of a portion of the stimulatory activity and all of the inhibitory activity from lymphocytes and monocytes, respectively, is suppressed by cytochalasin B. Accordingly, cytochalasin B treatment per se results in partial removal of burst stimulatory activity from LCM (compare results with LCM alone to those with dialyzed LCM, Fig 2C), and in unopposed stimulatory activity in MCM (compare results with MCM alone to those with dialyzed MCM, Fig 2D).

The capacity of cytochalasin B to block release of positive regulatory factors from lymphocytes correlated with the amount of cytochalasin B added (Fig 3). Here, half maximal suppression of regulator release occurred at a concentration of approximately 1 μg/mL. Because stimulatory activity was not fully removed from dialyzed LCM even at concentrations of 100 μg cytochalasin B per milliliter, the possibility that the release of a class of positive regulatory molecules is unaffected by cytochalasin treatment was investigated.

Antiproliferative effects of cytochalasin B are directed at the cell surface. The effects of cytochalasin B and colchicine on release of stimulatory activities in LCM-derived supernatants and pellets obtained after high speed centrifugation were measured. Figure 4 shows that incubation of lymphocytes with 10 μg cytochalasin B per milliliter partially removed burst stimulatory activity of dialyzed unseparated LCM (P < .05), and that this reduction could not be attributed to loss of active soluble molecules in LCM supernatant. In contrast, a similar amount of colchicine had no effect on the release of stimulatory molecules from lymphocytes (Fig 4). Similar results were obtained in tests of MCM supernatant (data not shown).

However, both the stimulatory activity expressed by membrane-derived, vesicle-rich LCM pellets and the inhibitory activity expressed by MCM pellets, were abolished by exposure to cytochalasin B (Fig 5). This was true in tests of pellets
added over a wide range of volumes and protein concentrations (Figs 5 and 6A). On the other hand, when vesicles exfoliated from untreated lymphocytes were subsequently incubated with cytochalasin B at a high concentration (100 \( \mu \)g/mL) and added to culture, there was no effect on stimulatory activity: 10 \( \mu \)g vesicles per milliliter incubated
with alpha medium alone enhanced erythroid burst formation by 243% ± 10%, while 10 μg vesicles per milliliter incubated with cytochalasin B stimulated burst formation by 236% ± 12% (P > .10).

To further assess an effect of cytochalasins on the expression of membrane-associated BPA, plasma membranes were prepared from untreated lymphocytes and lymphocytes exposed to cytochalasin B. As shown in Fig 6B, in contrast to membranes of untreated cells, membranes of cytochalasin treated cells expressed virtually no BPA (relative to cultures prepared with no added membrane; P > .10). Furthermore, attempts to solubilize BPA from membranes of cytochalasin B-treated lymphocytes were also unsuccessful (Table 1). The data indicate that expression of membrane-associated regulatory activities is profoundly impaired by exposure of mononuclear cells to cytochalasin B but not to colchicine. In contrast, there is no apparent effect of cytochalasin B on the release of soluble growth factors or on the expression of factors associated with membrane vesicles that are previously shed from untreated cells.

**Mechanism of cytochalasin effects on regulator release.** To determine how cytochalasins interfere with generation of membrane-associated regulatory activity, the effects of cytochalasin on mononuclear cell shedding of membrane vesicles were measured. Relative to unexposed cells, the amount of shed vesicular protein was increased two- to threefold in CM prepared in the presence of 10 μg cytochalasin B per milliliter. At the same time, the amount of protein associated with plasma membrane was decreased by approximately 50% by treatment of lymphocytes with cytochalasin B.

Next, changes in the distribution of actin between unpolymerized and filamentous pools were assessed in mononuclear cells incubated at 37°C for 24 hours without and with 10 μg cytochalasin B per milliliter. Whereas lysates of unexposed cells inhibited the activity of DNase I by 23%, lysates of cytochalasin B-exposed cells inhibited activity by 38% (ratio of inhibitory activity in lysates of treated v untreated cells is 1.65:1.00). Since monomeric actin (G-actin) binds to DNase I with greater affinity that does filamentous actin (F-actin), the data are consistent with the notion that cytochalasin B interfered with actin filament growth in these cells. When filamentous actin was depolymerized and total actin content was determined, lysates of cytochalasin B-treated cells were found to contain an average of 90% G-actin in two separate experiments (i.e., approximately 10% F-actin). In contrast, lysates of untreated cells contained an average of 70% G-actin in two studies, as determined by the DNase inhibition assay. This represents a significant increase in monomeric actin in cytochalasin B-exposed cells.

To investigate whether the inhibitory action of cytochalasins on growth factor release is mediated through an effect on glucose transport, we determined whether the release of stimulatory activity is suppressed by cytochalasin D, an agent that binds to the rapidly polymerizing end of actin filaments without affecting glucose transport sites. As shown in Fig 3, the effect of cytochalasin D was indistinguishable from that of cytochalasin B over a wide range of concentrations (P > .10 for each). Furthermore, addition of 0.5 mol/L or 1.0 mol/L D-glucose together with cytochalasin B did not alter the inhibitory action of this agent on growth factor release from lymphocytes (Table 2).

**DISCUSSION**

The importance of mononuclear cell surface-associated factors to hematopoietic progenitor proliferation has been emphasized. A recognized source of hematopoietic growth promoting molecules (or lymphokines) is the lymphocyte.

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**Table 1. Influence of Cytochalasin B on Stimulatory Activities in Octylglucoside Extracted Membranes and Shed Vesicles**

<table>
<thead>
<tr>
<th>Addition to Culture</th>
<th>Mean ± SE Bursts per 6 x 10⁶ Cells</th>
</tr>
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<tbody>
<tr>
<td>None</td>
<td>20 ± 2</td>
</tr>
<tr>
<td>LCM pellet</td>
<td></td>
</tr>
<tr>
<td>Unexposed</td>
<td>(1 μg/mL) 28 ± 1 (P &lt; .05)</td>
</tr>
<tr>
<td></td>
<td>(10 μg/mL) 44 ± 2 (P &lt; .05)</td>
</tr>
<tr>
<td>CyB-exposed</td>
<td>(1 μg/mL) 17 ± 1 (P &gt; .10)</td>
</tr>
<tr>
<td></td>
<td>(10 μg/mL) 17 ± 1 (P &gt; .10)</td>
</tr>
<tr>
<td>Lymphocyte membranes</td>
<td></td>
</tr>
<tr>
<td>Unexposed</td>
<td>(1 μg/mL) 21 ± 2 (P &gt; .10)</td>
</tr>
<tr>
<td></td>
<td>(10 μg/mL) 42 ± 2 (P &lt; .05)</td>
</tr>
<tr>
<td>CyB-exposed</td>
<td>(1 μg/mL) 17 ± 1 (P &gt; .10)</td>
</tr>
<tr>
<td></td>
<td>(10 μg/mL) 19 ± 1 (P &gt; .10)</td>
</tr>
</tbody>
</table>

Results in quadruplicate 125 μL fibrin clots are displayed. All cultures contained 2.0 IU/mL erythropoietin. LCM was prepared in the absence and presence of 10 μg CyB per milliliter. Plasma membranes and vesicles shed from unexposed and exposed lymphocytes were extracted with 30 mmol/L octylglucoside, as described previously, and added at the indicated protein concentrations (parentheses). P values are computed relative to results in cultures containing erythropoietin alone (none).

**Table 2. Effects of Cytochalasin B on Stimulatory Activity in the Presence and Absence of D-Glucose**

<table>
<thead>
<tr>
<th>Addition to Culture</th>
<th>Mean ± SE Bursts per 6 x 10⁶ Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>LCM without CyB</td>
<td>38 ± 3</td>
</tr>
<tr>
<td>or D-glucose</td>
<td></td>
</tr>
<tr>
<td>LCM plus CyB alone</td>
<td>17 ± 2 (P &lt; .05)*</td>
</tr>
<tr>
<td>D-glucose alone</td>
<td>(0.5 mol/L) 38 ± 4 (P &gt; .10)*</td>
</tr>
<tr>
<td>(1.0 mol/L) 39 ± 2 (P &gt; .10)*</td>
<td></td>
</tr>
<tr>
<td>CyB + D-glucose</td>
<td>(0.5 mol/L) 18 ± 1 (P &lt; .05)*</td>
</tr>
<tr>
<td>(1.0 mol/L) 17 ± 1 (P &gt; .10)*</td>
<td></td>
</tr>
</tbody>
</table>

Results in quadruplicate 125 μL fibrin clots containing 2.0 IU/mL erythropoietin alone (none) or erythropoietin plus 9% (vol/vol) LCM are displayed. CyB incubations were performed at the half maximal concentration of 5 μg/mL.

*P values are computed relative to results in cultures containing LCM without CyB or D-glucose.
†P values are computed relative to results in cultures containing LCM plus CyB.
Surface phenomena associated with lymphocyte function such as capping of membrane molecules appear to be directly linked to the cytoplasmic contractile apparatus. Conversely, pharmacologic manipulation of the cytoskeletal network triggers changes in surface organization, including segregation and translation of membrane proteins. Here, we have shown that fungal products capable of blocking polymerization of actin filaments abrogate the erythroid stimulating capacity of lymphocyte plasma membranes and of vesicles shed into liquid culture medium from the lymphocyte surface. Moreover, erythroid growth inhibitory activity of shed monocyte membrane vesicles is also abolished. In contrast, colchicine, an agent that blocks polymerization of tubulin into microtubules, has no effect on surface regulatory molecules. These results suggest that a functional interaction of filamentous actin with surface regulatory molecules exists in circulating mononuclear cells.

While it is still unknown precisely how cytochalasin B abolishes growth regulatory activity of surface components, results of several studies suggest that its effects are mediated by changes in the integrity of microfilaments. Our finding that deoxyribonuclease I activity is inhibited to a greater degree by lysates of cytochalasin B-treated cells than by lysates of untreated cells strongly suggests that the relative amount of G-actin in mononuclear cells is increased following exposure to this agent. This increase occurs over a period of time during which growth factor shedding into liquid culture medium occurs (ie., 24 hours). In addition, results of our studies with D-glucose suggest that the action of cytochalasin B on regulator expression is unrelated to alterations in sugar metabolism (Table 2). Furthermore, cytochalasin D, an agent that binds to high-affinity, motility related actin binding sites, also suppresses stimulatory activity expressed in LCM (Fig 3). Therefore, although cytochalasin B binds to sites related to sugar transport, its effects on erythroid growth factor expression appear to be independent of this event.

It was important to determine whether or not cytochalasin B-induced changes in growth regulator activity expressed by shed vesicles was due to a global effect on cell surface exfoliation. In contrast to their effects on shedding of vesicle-associated regulators, cytochalasins actually augmented vesiculation of membrane protein overall, without altering cell viability. This was accompanied by decreased membrane-associated protein. When corrected for protein concentration, the amount of growth stimulatory activity was diminished in both membranes and shed vesicles (Fig 6), suggesting that the association of regulatory molecules with membranes is selectively altered by cytochalasin treatment.

Our finding that no stimulatory activity was extractable with octylglucoside from either membranes or shed vesicles of treated cells (Table 1) supports this hypothesis.

Based on these findings, a hypothetical model of regulator release from mononuclear cells may be proposed (Fig 7). Accordingly, as erythroid growth regulatory molecules are synthesized and/or after being pooled, they are inserted into the plasma membrane as integral components. Ultimately, they are exfoliated from the cell surface in association with membrane-derived vesicles. This sequence of events is suggested by our previous reports showing that factor-rich vesicles are derived from the plasma membrane, that activity in shed vesicles is associated with vesicle surface rather than with intravesicular material, and that the chromatographic behavior of the growth factor extracted from plasma membranes is indistinguishable from that purified from shed vesicles. In the presence of cytochalasins, insertion of newly synthesized and/or pooled growth regulators into the membrane is impaired, resulting in loss of activity in both membranes and shed vesicles. Our finding that activity expressed by membrane vesicles from untreated lymphocytes is unaltered by subsequent treatment with cytochalasin B suggests that once inserted into the membrane, erythropoietic molecules are functionally stable with respect to the drug. On the other hand, disruption of microfilaments appears to result in transit of regulatory molecules in the soluble form across the membrane without anchorage in the membrane (Fig 7). This may explain our findings that (1) in contrast to its action on membrane-associated regulatory activity, cytochalasin B does not impair the expression of soluble regulatory activity (Figs 2C and 4); and (2) the capacity of monocytes to release a soluble stimulatory factor that has been previously described is likewise unaffected by treatment with cytochalasin B (Fig 2D). Therefore, according to this model, a critical concentration of F-actin in the cytoplasm is important for proper localization of erythroid growth factors to the plasma membrane. From this model one may predict that microfilament integrity is not important for synthesis, transcription, translation, or processing of such factors.

Results of our studies designed to evaluate cytoskeletal disruptors by direct addition to marrow culture demonstrate the complexity of such an approach to evaluate drug effects. Both cytochalasins and colchicine may be anticipated to exert antiproliferative action on all cell types since microtubule and microfilament fluorescence studies using anti-tubulin and anti-actin antibodies demonstrate that both tubulin and actin are actively involved in formation of the
mitotic spindle and the contractile ring during cell division. Thus, effects of cytoskeletal protein disruptors on growth factor production/release from accessory cells in the local environment of cultured bone marrow may not be discernible. In addition, it is unclear why the antiproliferative action of cytochalasins is observed at a slightly lower drug concentration than that required for suppression of membrane proteins. The results do suggest, however, that cell division is not required for growth regulator production, a hypothesis that is supported by the finding that mitogens are not required for growth factor production by mononuclear cells present in the circulation or bone marrow.

Functional interaction of surface receptor molecules and cytoskeletal proteins occurs in vitro during differentiation of many cell types. In addition, several growth factors whose receptors are located on the cell surface have been reported to directly regulate the architecture of cytoskeletal elements. However, little information is available concerning the role of cytoskeleton activity in maintaining a functional repertoire of surface membrane molecules. Here, we present data suggesting that changes in the distribution of actin between unpolimerized and filamentous pools may influence the organization and/or function of mononuclear cell surface growth regulatory molecules. The precise role of such molecules as messengers involved in hematopoietic cell to cell interactions is under investigation.

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Contractile proteins participate in release of erythroid growth regulators from mononuclear cells

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