Autocrine Activities of Basic Fibroblast Growth Factor: Regulation of Endothelial Cell Movement, Plasminogen Activator Synthesis, and DNA Synthesis

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Abstract. We have found that the spontaneous migration of bovine aortic endothelial cells from the edge of a denuded area in a confluent monolayer is dependent upon the release of endogenous basic fibroblast growth factor (bFGF). Cell movement is blocked by purified polyclonal rabbit IgG to bFGF as well as affinity purified anti-bFGF IgG and anti-bFGF $F(ab')_2$ fragments. The inhibitory effect of the immunoglobulins is dependent upon antibody concentration, is reversible, is overcome by the addition of recombinant bFGF, and is removed by affinity chromatography of the antiserum through a column of bFGF-Sepharose. Cell movement is also reversibly inhibited by the addition of protamine sulfate and suramin; two agents reported to block bFGF binding to its receptor. The addition of recombinant bFGF to wounded monolayers accelerates the movement of cells into the denuded area. Transforming growth factor β which has been shown to antagonize several other effects of bFGF also inhibits cell movement. The anti-bFGF IgG prevents the movement of bovine capillary endothelial cells, BHK-21, NIH 3T3, and human skin fibroblasts into a denuded area. Antibodies to bFGF, as well as suramin and protamine sulfate also suppress the basal levels of plasminogen activator and DNA synthesis in bovine aortic endothelial cells.

Basic fibroblast growth factor (bFGF)[1] is a potent mitogen for a number of cell types including fibroblasts, endothelial cells, chondrocytes, adrenal cortical cells, and granulosa cells (see Gospodarowicz et al., 1987 for review). In addition to being mitogenic, bFGF affects a variety of activities in cultured cells including protease production (Moscatelli et al., 1986*b*; Presta et al., 1986) and chemotaxis (Moscatelli et al., 1986*b*; Presta et al., 1986), as well as supporting the maintenance and differentiation of neurons (Hatten et al., 1988; Morrison et al., 1986; Walicke et al., 1986). bFGF is synthesized in vitro by a large number of cell types including fibroblasts (Moscatelli et al., 1986*a*), endothelial cells (Moscatelli et al., 1986*a*; Vlodavsky et al., 1987*a*, *b*; Schweigerer et al., 1987*b*), retinal pigment cells (Schweigerer et al., 1987*a*), granulosa cells (Neufeld et al., 1987), glial cells (Hatten et al., 1988), adrenal cortical cells (Schweigerer et al., 1987*c*), and cells from a variety of tumor cell lines (Moscatelli et al., 1986*a*; Klagsbrun et al., 1986, Schweigerer et al., 1987*d*).

Since many cells that synthesize bFGF are capable of responding to bFGF through interaction with high affinity plasma membrane receptors, it is reasonable to hypothesize that bFGF acts as an autocrine factor in the regulation of specific cellular functions. However, the nucleotide sequence of bFGF cDNA indicates that the protein is synthesized without a signal sequence (Abraham et al., 1986*a*, *b*; Kurokawa et al., 1987; Sommer et al., 1987) and, therefore, lacks the structural features normally required for protein secretion. Consistent with this is the observation that cells that synthesize bFGF appear to release little or no growth factor into the medium (Presta et al., 1986; Vlodavsky et al., 1987*b*). Thus, a growth factor that has the features of an autocrine hormone appears to be a cytoplasmic nonsecretory protein.

In this study we report that the endogenous production of bFGF is required for the movement of bovine endothelial cells. Our results indicate that endothelial cell migration, as well as fibroblast migration, is regulated by bFGF released by the cells themselves. In addition, the basal level of synthesis of the protease plasminogen activator (PA) and the basal level of DNA synthesis in bovine aortic endothelial (BAE) cells appear to be determined by the release of endogenous bFGF, as antibodies to bFGF suppress both of these activities. These effects represent the first examples of natural autocrine roles of bFGF.

1. *Abbreviations used in this paper:* BAE, bovine aortic endothelial; BCE, bovine capillary endothelial; bFGF, basic fibroblast growth factor; PA, plasminogen activator; rbFGF, recombinant basic fibroblast growth factor; TGFβ, transforming growth factor β.

Materials and Methods

Cell Culture

BAE cells and bovine capillary endothelial (BCE) cells were isolated and grown as described previously (Gross et al., 1982). Briefly, the cells were grown on gelatin-coated dishes in alpha minimal essential medium contain-

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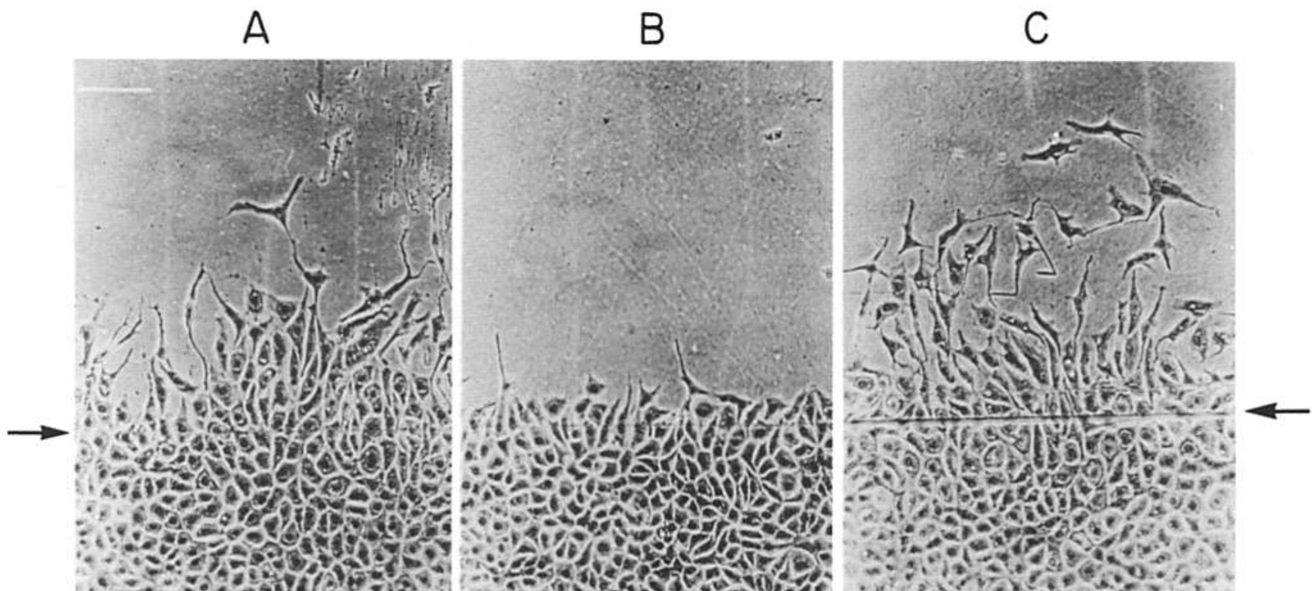

Figure 1. BAE cell migration in the presence of anti–bFGF IgG. Confluent monolayers of BAE cells were wounded with a razor blade as described in Materials and Methods. The cells were incubated overnight in the indicated media, fixed, stained, and photographed. *A*, control; *B*, anti–bFGF IgG (200 μg/ml); *C*, nonimmune IgG (200 μg/ml). The arrows point to the original edge of the wound. Bar, 100 μm.

ing 10% (BAE) or 5% (BCE) calf serum. Cells were used between passages 12–18. BHK-21 cells were the gift of Dr. C. Basilico (New York University Medical School). BHK-21, NIH 3T3, and human embryonic skin fibroblasts were grown in DME in 10% calf serum.

bFGF

Placental bFGF was prepared and assayed as described by Moscatelli et al. (1986*b*), and Presta et al. (1986). Recombinant bFGF (rbFGF) was a generous gift from Synergen, Inc. (Boulder, CO).

Anti–Human Placental bFGF Antibodies

Polyclonal antibodies against human placental bFGF were raised in rabbits as described (Joseph-Silverstein et al., 1988). These antibodies recognize human placental bFGF but no other heparin-binding proteins in a Western blot assay or in dot blot analysis. DEAE Affi-Gel Blue (Bio-Rad Laboratories, Cambridge, MA) chromatography was used to prepare IgG fractions according to the manufacturer's instructions. $F(ab')_2$ fragments were prepared by pepsin digestion of purified IgG in acetate buffer at pH 4.5 (Stanworth and Turner, 1978). The digestion was stopped by raising the pH to 8.0. The $F(ab')_2$ fragments were isolated by gel filtration through a 50-cm column of Sephadex G 150 in Tris-HCl buffer (pH 7.7).

Wound Assays

Confluent monolayers of endothelial cells were wounded with a razor blade as described by Burk (1973). After wounding, the cultures were washed with PBS and further incubated in alpha minimal essential medium containing 0.1% gelatin. Control cultures, antibody-treated cultures, and cultures containing suramin (Mobay Chemical Corp., Pittsburgh, PA), protamine sulfate (Sigma Chemical Co., St. Louis, MO), rbFGF, and/or transforming growth factor β (TGFβ) (R&D Systems, Inc., Minneapolis, MN) were incubated at 37° for 16–20 h. After the incubation, the cells were fixed with absolute methanol and stained with Giemsa. Migration was quantitated by counting the number of cells in successive (7) 125-μM sections from the wound edge. Cells were counted at 100× magnification using a light microscope with an occular grid. The values represent the mean from four different fields. The percent inhibition was determined by dividing the number of cells that had moved from the wound edge in the experimental cultures by the number of cells that had moved from the wound edge in the control cultures, multiplying this number by 100, and subtracting the product from 100.

Assays for PA Induction

Confluent cultures of BAE cells were incubated overnight under the various conditions indicated. After incubation, the BAE cells were washed twice with PBS and extracted with 0.5% Triton X-100 in 0.1 M Tris-HCl, pH 8.1. The resulting cell extracts were assayed for PA activity in the fibrin plate assay (Gross et al., 1982).

Thymidine Incorporation Assay

Confluent monolayers of BAE cells were maintained for 7 d in alpha minimal essential medium with 5% calf serum. The medium was then replaced with fresh alpha minimal essential medium with 1% calf serum with or without 0.75 mM suramin. After 24-h incubation, the cultures were washed twice with PBS containing 0.75 mM suramin and incubated for a further 24 h in DME containing 0.5% calf serum plus the additions indicated. During the second 24-h period, the cells were incubated with [^{3}H]thymidine (1 uCi/ml of [Methyl-^{3}H]thymidine, 6.7 Ci/mMole; New England Nuclear, Boston, MA) and incorporation of [^{3}H]thymidine into TCA precipitable material after this period was determined by liquid scintillation counting (Presta et al., 1986). Under these conditions there is no increase in cell number and thymidine incorporation is $<3\%$ of that observed in growing control cells.

Results

The migration of endothelial cells is one of the critical features of neovascularization and wound repair, two processes thought to involve bFGF. Therefore, we examined the role of bFGF in the movement of BAE cells from a wound edge in vitro. The wound was made by removing a patch of cells from a confluent monolayer with a razor blade. To avoid the possible effects of serum factors, all experiments were conducted in the absence of serum. Under these conditions, BAE cells rapidly moved from the edge of the wound into the open area (Fig. 1 *A*). The inclusion of anti–bFGF IgG in the medium inhibited the migration of the cells into the denuded area (Fig. 1 *B*), while the inclusion of nonimmune IgG had no effect on cell movement (Fig. 1 *C*). Similar results were

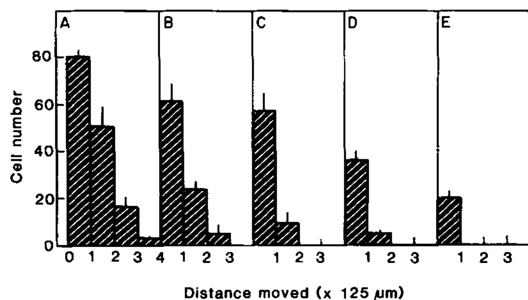

Figure 2. Quantitative dose-dependent inhibition of cell migration by anti-bFGF IgG. Confluent cultures of BAE cells were wounded as described in Materials and Methods. The cells were incubated with the indicated additions for 20 h, fixed, and stained. Migration was quantitated by observing the cells with a grid marked in increments of 125 μM. The number of cells within each 125-μM area in four fields was counted using the original mark made by the razor blade as the origin. The results are presented as the average number of cells per field. *A*, control; *B*, anti-bFGF IgG (25 μg/ml); *C*, anti-bFGF IgG (50 μg/ml); *D*, anti-bFGF IgG (100 μg/ml); *E*, anti-bFGF IgG (200 μg/ml). The total number of cells that moved from the edge of the wound was (*A*) 149, (*B*) 90, (*C*) 67, (*D*) 41, and (*E*) 20.

obtained when this experiment was performed in the presence of serum (data not shown). Under these conditions round, refractile cells undergoing mitosis were present in the region of cell movement. Cells undergoing mitosis were not seen in the nonwounded regions. In the absence of serum no mitotic cells were apparent in the area of migration.

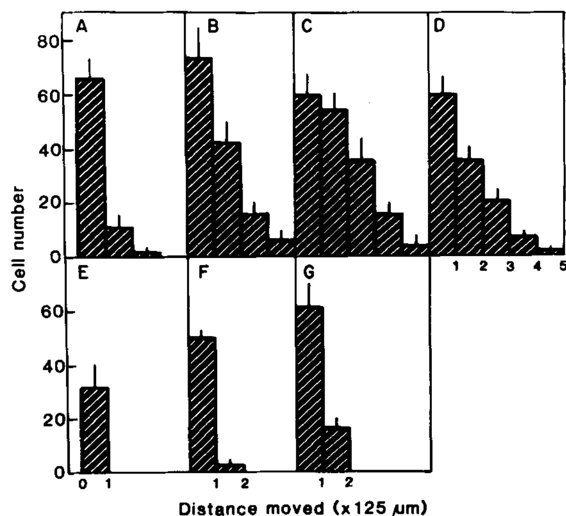

Figure 3. Potentiation of BAE cell migration by recombinant bFGF. Confluent monolayers of BAE cells were wounded as described in Materials and Methods. The cells were incubated overnight with the indicated additions and migration was quantitated as described in the legend to Fig. 2. *A*, control; *B*, recombinant bFGF (1 ng/ml); *C*, recombinant bFGF (10 ng/ml); *D*, recombinant bFGF (100 ng/ml); *E*, anti-bFGF IgG (200 μg/ml); *F*, anti-bFGF IgG (200 μg/ml) plus recombinant bFGF (10 ng/ml); *G*, anti-bFGF IgG (200 μg/ml) plus recombinant bFGF (100 ng/ml). The total number of cells that moved from the edge of the wound was (*A*) 78, (*B*) 139, (*C*) 164, (*D*) 125, (*E*) 32, (*F*) 52, and (*G*) 79.

To quantitate the effect of the anti-bFGF antibodies on BAE cell movement, cells were fixed and stained at the completion of the experiment, and the distance moved measured in increments of 125 μm (Fig. 2). The antibody-induced inhibition of cell movement occurred in a dose-dependent manner with 40% inhibition detected at an IgG concentration of 25 μg/ml (Fig. 2 *B*) and 87% inhibition at an IgG concentration of 200 μg/ml (Fig. 2 *D*).

Several control experiments were performed to verify the specificity of the antibody. Nonimmune IgG had no effect on cell movement nor did irrelevant antibodies such as anti-fibronectin IgG or anti-BSA IgG (data not shown). The addition of rbFGF potentiated BAE cell movement (Fig. 3, *B*, *C*, and *D*) from 60 to 110% depending upon the dose. The addition of rbFGF to medium containing anti-bFGF IgG reversed the inhibitory effect of the immune IgG (Fig. 3, *F* and *G*). Chromatography of the anti-bFGF IgG preparation through a column of rbFGF-Sepharose removed the inhibitory activity (Fig. 4, *D* and *E*). Moreover, the antibodies eluted from the rbFGF-Sepharose column retained their inhibitory activity at a significantly lower concentration (Fig. 4 *F*). These experiments indicated that the inhibition of BAE migration was a direct consequence of the neutralization of bFGF by the antiserum.

Suramin, a polyanionic trypanocidal drug, and protamine sulfate, a small basic protein, have been reported to block the interaction of bFGF with its receptor (Coffey, Jr., et al., 1987; Neufeld and Gospodarowicz, 1987). Both suramin and protamine sulfate also inhibited BAE cell movement (Table I). The effects of both compounds were reversible (Table I). Heparin, another molecule that interacts with bFGF but does not affect bFGF binding to its receptor (Moscatelli, 1987), neither stimulated nor inhibited cell movement (Table I).

Previous experiments by Frater-Schroeder et al. (1986) and Baird and Durkin (1986), demonstrated that TGFβ can block the mitogenic effect of bFGF, and Saksela et al. (1987) have shown that TGFβ suppresses the induction of PA in BCE cells by bFGF. Therefore, we monitored the effect of TGFβ on BCE movement (Table I). TGFβ at a concentration

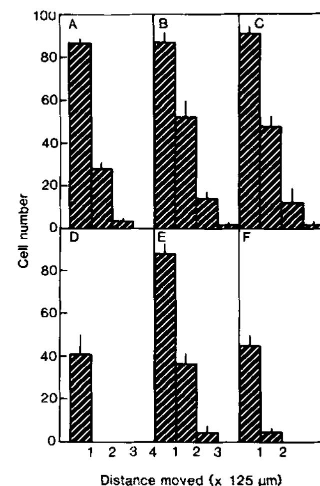

Figure 4. Effect of affinity purified anti-bFGF antibodies. Confluent monolayers of BAE cells were wounded as described in Materials and Methods. The cells were incubated overnight with the indicated additions and migration was quantitated as described in the legend to Fig. 2. *A*, control; *B*, nonimmune IgG (200 μg/ml); *C*, nonimmune IgG after passage through a column of bFGF-Sepharose (200 μg/ml); *D*, anti-bFGF IgG (200 μg/ml); *E*, anti-bFGF IgG after passage through a column of bFGF-Sepharose (200 μg/ml); *F*, IgG bound to bFGF-Sepharose and eluted with 0.1 M glycine, pH 2.5 (2 μg/ml). The total number of cells that moved from the edge of the wound was (*A*) 118, (*B*) 154, (*C*) 151, (*D*) 41, (*E*) 128, and (*F*) 48.

Table I. Effect of Suramin and Other Agents on BAE Cell Migration

Addition	Migration (percent of control)
–	100
Nonimmune IgG (200 μg/ml)	98
Immune IgG (200 μg/ml)	27
Suramin (0.5 mM)	4
Suramin (0.5 mM) removed after 20 h	110
Protamine (0.5 μg/ml)	11
Protamine (0.5 μg/ml) removed after 20 h	84
Heparin (10 μg/ml)	97
TGFβ (2 ng/ml)	49
rbFGF (20 ng/ml)	170
TGFβ (2 ng/ml) plus rbFGF (20 ng/ml)	111

Confluent monolayers of BAE cells were wounded as described in Materials and Methods. The cells were incubated for 20 h in media containing the listed additions, fixed, and cell migration was quantitated. In the case of suramin and protamine, after a 20-h incubation, the drugs were removed from the cultures by washing, the medium was replaced with control medium, and the cells were fixed and counted after a further 20-h incubation. In the control cultures, 100% represents a total of 118 cells that had moved from the edge of the wound or in the case of the TGFβ and rbFGF experiments, 176 cells.

of 2 ng/ml inhibited BAE cell movement by 51% (Table I). This result confirms an earlier report of Heimark et al. (1986) describing the inhibition of BAE cell migration by TGFβ. The inhibitory effect of TGFβ was reversed by the

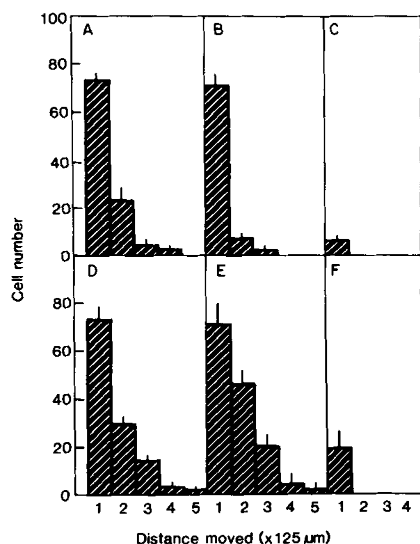

Figure 5. Effect of suramin incubation on BAE cell migration. Confluent cultures of BAE cells were incubated for 2 h with 0.5 mM suramin, wounded as described in Materials and Methods, washed twice with PBS containing 0.5 mM suramin and incubated overnight with the indicated additions. Cell migration was quantitated as described in the legend to Fig. 2. *A*, control; *B*, suramin removed after the PBS wash; *C*, suramin present throughout; *D*, suramin removed after an overnight incubation, and the culture incubated in control medium for an additional 20 h; *E*, suramin removed after overnight incubation, replaced by nonimmune IgG (200 μg/ml), and the culture incubated for an additional 20 h; *F*, suramin removed after overnight incubation, replaced by anti-bFGF IgG (200 μg/ml), and the culture incubated for an additional 20 h. The total number of cells that moved from the edge of the wound was (*A*) 102, (*B*) 80, (*C*) 6, (*D*) 126, (*E*) 141, and (*F*) 19.

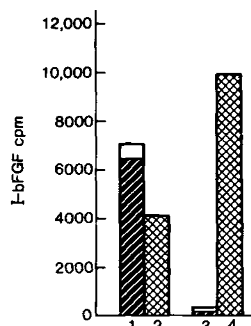

Figure 6. Removal of bFGF from high and low affinity sites by suramin. Confluent cultures of BAE cells were incubated with 2.5 ng/ml of ^{125}I-bFGF (542 cpm/fmol) for 2 h at 4°C. The cultures were quickly washed twice with ice-cold PBS and incubated with either control medium or medium containing suramin (0.5 mM) for 2 h at 4°C. At the end of 2 h the medium was removed and ^{125}I counted. The radioactivity remaining with the cell layer was analyzed for ^{125}I-bFGF binding to both high and low affinity sites as described by Moscatelli (1987). *Column 1*, radioactivity remaining associated with cells in control cultures; *column 2*, radioactivity in the medium after a 2-h incubation of control cultures; *column 3*, radioactivity associated with cells after a 2-h incubation with suramin (0.5 mM); *column 4*, radioactivity in the medium after a 2-h incubation with suramin (0.5 mM). ▭, high affinity binding; ▨, low affinity binding; ▩, radioactivity in culture fluid.

simultaneous addition of sufficient rbFGF with the TGFβ (Table I).

These results indicate that the release of bFGF is required for the movement of BAE cells from a wound edge. As bFGF lacks a signal sequence, the mechanism for its release from cells is unclear. It is possible that the bFGF responsible for BAE migration was liberated only from BAE cells injured during wounding. Conversely, bFGF may be continuously released by BAE cells under the culture conditions. We attempted to distinguish between these two alternatives by the following experiment. BAE monolayers were wounded in the presence of 0.5 mM suramin to prevent any bFGF released from binding to the cells (Fig. 5). After wounding, the cultures were washed twice with PBS containing suramin to remove released bFGF and incubated for 20 h in the presence (Fig. 5 *C*) or absence (Fig. 5 *B*) of suramin. When suramin was present continuously, there was no significant cell movement from the wound edge (compare Fig. 5 *A* with *C*). However, when suramin was present only during the wounding and PBS wash, and subsequently removed, only a slight inhibition (20%) of cell movement was detected (Fig. 5 *B*). The effect of continous exposure to suramin was reversible. If the suramin was removed after a 20-h exposure, cell migration resumed during a subsequent 20-h period (Fig. 5 *D*). The resumption of cell movement was unaffected by the inclusion of nonimmune IgG (Fig. 5 *E*) but was inhibited by immune IgG (Fig. 5 *F*).

While it is possible that some bFGF released by the act of wounding may have remained bound to the matrix, this is unlikely since suramin removes bFGF bound to the matrix low affinity binding sites as well as blocking bFGF from binding to its high affinity plasma membrane receptor. This was shown by exposing BAE cultures to ^{125}I-bFGF for 2 h at 4°C to permit binding to both high and low affinity binding sites (Fig. 6, column *1*; Moscatelli, 1987). The cells were then incubated for 2 h in bFGF-free medium. During this time, ~36% of the cell-associated radioactivity reequilibrated into the culture medium (Fig. 6, column *2*). Of the ^{125}I-bFGF that remained cell associated, ~7% was bound to high affinity receptors, while 93% was bound to low affinity binding sites probably representing heparan sulfate

Table II. Effect of Anti-bFGF IgG on the Migration of Other Cell Types

Cell type	Addition	Migration (percent of control)
BHK-21	–	100
BHK-21	50 μg/ml IgG	0
BCE	–	100
BCE	200 μg/ml IgG	6
NIH 3T3	–	100
NIH 3T3	200 μg/ml IgG	25
HES	–	100
HES	200 μg/ml IgG	22

Confluent monolayers of cells were wounded as described in Materials and Methods. Increasing amounts of anti-bFGF IgG were added to the experimental cultures, the cells were incubated overnight, the monolayers were fixed, and the number of cells that had migrated from the edge of the wound were counted. The results for the maximum amount of IgG used are presented as a percentage of the control. The number of cells that migrated in the control cultures were 64 for BHK-21, 114 for BCE cells, 20 for NIH 3T3, and 36 for human embryonic skin (HES).

molecules (Fig. 6, column *1*). If cells preincubated with ^{125}I-bFGF were subsequently incubated with bFGF-free medium containing 0.5 mM suramin for 2 h, the amount of ^{125}I-bFGF that remained associated with the cells was reduced to 4% of the total initially bound (Fig. 6, columns *3* and *4*). This was evenly distributed between high and low affinity binding sites. Thus, a 2-h exposure to suramin dissociated >76% of the bFGF bound to high affinity sites and 98% bound to low affinity sites. These results suggest that suramin can not only block bFGF-receptor binding but also can remove bFGF already bound to cell receptors and matrix. Therefore, after an overnight incubation in suramin-containing medium (Fig. 5 *D*), very little bFGF would have remained complexed to cells or their matrices, and the resumption of cell movement after removal of the suramin must have been due to the continuous release of bFGF by the cells.

The bFGF-dependence of cell migration was not unique to BAE cells. BCE, NIH 3T3, BHK-21, and human embryonic skin fibroblast cell movement were all blocked by anti-bFGF IgG (Table II). With the exception of BCE cells, the number of cells that migrated was significantly lower than that observed with BAE cells. The inhibition of BHK-21 cell movement by antibodies to bFGF was surprising since these cells have been reported not to produce bFGF. An examination of the BHK-21 cells revealed, however, that the strain used in our study did contain low levels of bFGF (Sato, Y., and M. Renko, unpublished observation). Consistent with this observation, the concentration of antibody required for inhibition of BHK-21 cell movement was significantly less than that required for the inhibition of BAE cells that contain relatively high amounts of bFGF.

Since bFGF stimulates the synthesis of PA by endothelial cells (Moscatelli et al., 1986*b*; Presta et al., 1986; Saksela et al., 1987), we next examined the role of endogenous bFGF in regulating basal PA levels. Cultures of BAE cells were incubated in serum-free medium either with no additions or with immune or nonimmune rabbit IgG. As can be seen from Fig. 7, exposure of the cells to immune IgG suppressed the level of PA activity by 85%, while the inclusion of nonimmune IgG had little effect. Suramin and protamine sulfate also caused a decrease in PA levels (data not shown). The ob-

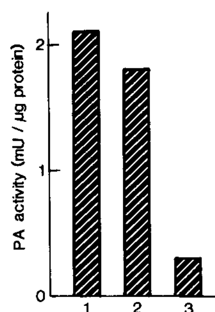

Figure 7. Inhibition of plasminogen activator production by anti-bFGF IgG. Cultures of confluent BAE cells were incubated with the indicated additions overnight, and the PA level of the cells were measured in cell extracts. *Column 1*, control cultures; *column 2*, nonimmune IgG (200 μg/ml); *column 3*, anti-bFGF IgG (200 μg/ml).

served decrease in PA activity represented an actual diminution in the amount of PA present as monitored by analysis of PA levels after SDS-PAGE and zymography (data not shown). In addition, no substantial increase in the amount of PAI-1, an inhibitor of PA synthesized by endothelial cells (van Mourik et al., 1984), was observed under these conditions (data not shown). Similar results on the suppression of basal PA production by anti-bFGF IgG have been obtained with BCE cells. Thus, in addition to cell migration, the basal level of PA synthesis in bovine endothelial cells may be regulated by the release of endogenous bFGF.

A similar experiment was performed to monitor the effect of anti-bFGF IgG on DNA synthesis by confluent BAE cells maintained in low serum. Under the conditions described in Materials and Methods, the basal level of [^{3}H]thymidine incorporation was not suppressed when anti-bFGF IgG was added to cultures of confluent BAE cells in 0.5% serum-containing medium (data not shown). This result is similar to that reported earlier by Schweigerer et al. (1987*b*) using BCE cells and an antiserum to bFGF. Since suramin removes bFGF from matrix and can be applied to the cultures in relatively high amounts, we treated confluent serum-starved BAE cells with 0.75 mM suramin to displace bound bFGF and measured [^{3}H]thymidine incorporation (Fig. 8, columns *1* and *3*). This treatment decreased DNA synthesis by ~86%. The effect was reversible as removal of suramin after a 24-h exposure to the drug resulted in a recovery of DNA synthesis during a subsequent 24-h incubation (Fig. 8, column *2*). If, after 24 h of exposure, suramin was removed and replaced with anti-bFGF IgG, there was no recovery of [^{3}H]thymidine incorporation (Fig. 8, column *5*). If nonim-

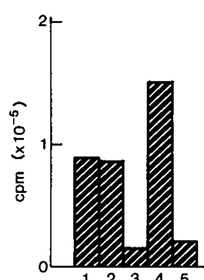

Figure 8. Suppression of DNA synthesis by anti-bFGF IgG. Confluent cultures of BAE cells were treated with the various regimens described below and incubated in medium containing [^{3}H]thymidine (1 uCi/ml) for 24 h. After incubation, the cells were washed, and [^{3}H]thymidine incorporation into TCA-insoluble material was measured as described in Materials and Methods. *Column 1*, control cells; *column 2*, suramin (0.75 mM) incubation overnight followed by removal of suramin and a 24-h incubation in control medium; *column 3*, incubation with suramin throughout; *column 4*, incubation with suramin overnight followed by a 24-h incubation with nonimmune IgG (200 μg/ml); *column 5*, incubation with suramin overnight followed by a 24-h incubation with anti-bFGF IgG (200 μg/ml).

mune IgGs were added, DNA synthesis recovered to a level 50% above that of the control (Fig. 8, column *4*). This increase in DNA synthesis to levels above the control may be the result of contaminating growth factors in the IgG preparation. The effect of the suramin anti-bFGF IgG treatment on BAE cells was similar for both confluent and sparse cultures (data not shown). These results demonstrate that the basal level of DNA synthesis in BAE confluent serum-starved cultures can be regulated by the release of endogenous bFGF.

Discussion

The results presented here demonstrate that the release of bFGF by BAE cells affects their movement and their basal levels of PA and DNA synthesis. We have previously reported that the release of bFGF from glial cells is important for the maintenance of cultured neurons (Hatten et al., 1988). In those experiments, bFGF release by glial cells in cocultures of glia and neurons was required for both the maintenance and differentiation of the neurons. Those experiments demonstrated that bFGF can function as a paracrine hormone. In the experiments described in this paper, endothelial cell functions were stimulated by endogenous bFGF demonstrating that bFGF can also act as an autocrine factor.

While our experiments appear to be the first documented example of an autocrine activity of bFGF, at least one earlier report described effects that may represent the autocrine activity of bFGF. Gospodarowicz et al. (1976) described an experiment in which endothelial cells seeded at low density did not survive in the absence of serum unless supplemented with crude FGF, while endothelial cells at high density survived in the absence of added serum or growth factor. At the time this experiment was performed, it was not appreciated that endothelial cells synthesize high levels of bFGF. In retrospect these results can be interpreted as evidence for autocrine conditioning of the culture medium with bFGF at high cell density, with insufficient amounts of bFGF accumulating at low cell density.

Baird et al. (1988) have recently shown that certain bFGF peptides may act as antagonists for the basal cell growth of BAE cells. These results are consistent with our observations on the role of endogenous bFGF on the BAE phenotype.

After this work was completed, two papers appeared indicating that when cells were transfected with high expression plasmids for bFGF, changes in the cell phenotype could be observed (Sasada et al., 1988; Rogelj et al., 1988). In one case the observed morphological change was slightly reverted upon the inclusion of anti-bFGF antibodies (Sasada et al., 1988). Therefore, over-expression of bFGF may also have profound effects on cells.

The migratory nature of cells at the edge of a wounded monolayer was observed over 18 years ago. The nature of the stimulus for this response has been explored since that time and a number of explanations of this phenomenon have been put forth, including alterations in the matrix (Stoker and Piggott, 1974), the activity of specific cell migration factors (Burk, 1973), and the action of plasmin (Ossowski et al., 1973, 1975). Since bFGF is believed to initiate endothelial cell migration during neovascularization and wound healing, we examined the role of bFGF in cell migration in vitro. While the chemotactic activity of bFGF has been described (Moscatelli et al., 1986*b*; Presta et al., 1986), the observed requirement for the release of endogenous bFGF for cell migration was unexpected.

These results, however, confirm a number of earlier reports and provide an interpretation for certain unexplained observations. For example, Heimark et al. (1986) described the inhibitory effect of TGFβ on movement and mitosis in wounded monolayers of BAE cells. Their observations, as well as the observations of a number of groups that TGFβ blocks the action of bFGF (Baird and Durkin, 1986; Frater-Schroder et al., 1986; Saksela et al., 1987), are consistent with a requirement for endogenous bFGF for cell movement. In the experiments of Heimark et al. (1986), the inhibitory effect of TGFβ was lost after 24 h. This is in agreement with the finding of Saksela et al. (1987) that the suppressive effect of TGFβ on bFGF-induced endothelial cell PA synthesis lasts for only 24 h. Thus, in the experiments of Heimark et al., the initial TGFβ-induced inhibition of cell migration was probably neutralized after 24 h by the stimulatory effect of bFGF released by the endothelial cells.

bFGF stimulates the synthesis of PA in BAE cells. A possible involvement of PA in the migratory process is consistent with a number of other observations. Earlier work by Ossowski et al. (1973, 1975) demonstrated that plasmin contributed to cell migration. Morioka et al. (1987) reported that in keratinocyte monolayers wounding is followed by the expression of PA, and presumably the formation of plasmin, by cells at the edge of the wound. Recently Pepper et al. (1987) demonstrated a similar stimulation of urokinase production at the edge of a wound in BAE cultures. Therefore, it will be of interest to determine if antibodies to bFGF prevent the induction of PA synthesis at the edge of a wound and if this blocks migration. However, an alternative explanation is that bFGF acts as a motility factor via its adhesive interactions described by Baird et al. (1988).

Perhaps, the most intriguing question raised by these experiments is the mechanism by which bFGF reaches the extracellular space. bFGF has no signal sequence and normally appears to be released from cells in limited amounts. It has been suggested that bFGF release occurs after cell death or damage. While this may be true, it is difficult to prove under normal culture conditions. Given the level of bFGF in BAE cells, ~25,000 molecules per cell, and the number of high affinity receptors per BAE cell, 3,000 (D. Moscatelli, personal communication), the death of a few cells would release sufficient growth factor to yield a response. While wounding may have caused the release of bFGF due to cell death, the reversible effects of suramin in wounding experiments, coupled with the ability of anti-bFGF IgG to modulate the synthesis of PA and DNA under conditions of minimal perturbation of the cells, strongly suggest that bFGF is continuously released in BAE cultures. The mechanism of bFGF release from BAE cells and the control of its extracellular activity are currently being explored.

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