Production of scatter factor by ndk, a strain of epithelial cells, and inhibition of scatter factor activity by suramin

JOSEPHINE C. ADAMS[1,*], ROBERT A. FURLONG[2,†] and FIONA M. WATT[1]

[1]*Keratinocyte Laboratory, Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London, WC2A 3PX, UK*
[2]*ICRF Laboratory and Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QP, UK*

*Present address: Room 304, Thorn Building, Department of Pathology, Brigham and Women's Hospital, 75 Francis Street, Boston, MA 02115, USA
†Present address: ICRF Cell Interactions Laboratory, Department of Medicine, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, UK

Summary

ndk are a strain of human epidermal keratinocytes that do not undergo terminal differentiation and which grow as single cells rather than compact colonies. We show that ndk are motile and secrete an epithelial scatter factor that has the same biochemical and immunological properties as the scatter factor previously purified from *ras*-transformed 3T3 fibroblasts. We have found that suramin, a polyanionic detergent, will reverse the activity of scatter factor from either cell type in the standard MDCK activity assay. When added to ndk cultures, suramin causes the cells to grow in coherent patches. This morphological change is accompanied by alterations in the distribution of actin and integrins, but not by stratification or terminal differentiation. The effect is reversed upon removal of suramin. We propose that the motile phenotype of ndk is due, at least in part, to autocrine production of scatter factor and that suramin may be useful for further studies of scatter factor binding to the cell surface.

Key words: non-differentiating keratinocytes, scatter factor, suramin, integrins, cytoskeleton.

Introduction

The epidermis is a tissue composed of multiple layers of epithelial cells, termed keratinocytes. Under normal conditions, proliferative cells are found in the basal layer; cells that become committed to terminal differentiation migrate out of the basal layer and undergo a series of changes in phenotype, which constitute the programme of terminal differentiation, as they move towards the tissue surface (Watt, 1989). In addition to upward migration, keratinocytes have the capacity for lateral migration, during wound healing (Colvin, 1989). Keratinocytes in culture can also migrate upwards and laterally: they form stratified colonies that expand by outward migration of cells at the periphery (Green, 1980). In culture, lateral migration is stimulated by EGF and TGFα (Barrandon and Green, 1987) and by scatter factor (Stoker *et al.* 1987).

Scatter factor is a polypeptide factor that was identified by its ability to cause dispersal of epithelial cell colonies. The MDCK cell line was found to be a particularly sensitive target cell and has been used in a bioassay for the factor (Stoker and Perryman, 1983, 1985; see Fig. 1A,B, below). To date, scatter factor has been found to be produced by fibroblastic and vascular smooth muscle cells (Stoker *et al.* 1987; Rosen *et al.* 1989). Purified murine scatter factor has a relative molecular mass of 62×10^3 and consists of two subunits of $57\times10^3 M_r$ and $30\times10^3 M_r$ held together by disulphide bonds (Gherardi *et al.* 1989). Recently, the $30\times10^3 M_r$ subunit has been shown to have amino-terminal protein sequence homology with the smaller subunit of human hepatocyte growth factor, a platelet-derived polypeptide that is mitogenic for primary hepatocytes (Gherardi and Stoker, 1990; Nakamura *et al.* 1990). Scatter factor is produced by the J2–3T3 cells used as feeder cells in keratinocyte cultures and may therefore contribute to the lateral motility of basal keratinocytes (Stoker *et al.* 1987).

ndk (non-differentiating keratinocytes) are a strain of keratinocytes that are unable to undergo terminal differentiation (Adams and Watt, 1988). The cells migrate laterally, but do not stratify and therefore grow as a monolayer. In morphology, the cells appear migratory: they have areas of ruffled membrane and many cells display spread 'head' and thin 'tail' regions of cytoplasm. Since cell growth shows an absolute requirement for EGF in the culture medium, the migratory phenotype does not appear to be due to autocrine stimulation by EGF or TGFα (Adams and Watt, 1988).

Here we report that ndk produce scatter factor and that suramin, a polyanionic detergent that can dissociate various polypeptide growth factors from their receptors (Hosang, 1985; Coffey *et al.* 1987), will abrogate the activity of scatter factor in the standard MDCK bioassay. When added to ndk cultures, suramin causes the cells to grow in compact colonies and to undergo alterations in the distribution of actin filaments and integrins. Our results

provide an example of autocrine stimulation by scatter factor and indicate that suramin may provide a useful tool for further experiments on scatter factor action.

Materials and methods

Materials

Suramin (Germanin®, Bayer 205) was kindly provided by Bayer UK Ltd, Newbury, Berks. A 100 mg ml^{-1} stock solution was stored at −20 °C. Texas Red-conjugated phalloidin was purchased from Sigma Chemical Co., Dorset, UK.

Antibodies

The rabbit antiserum to involucrin (Dover and Watt, 1987) and the rabbit antiserum to keratinocyte peanut lectin-binding glycoproteins (anti-PNA-gp) (Morrison *et al.* 1988) have been described previously.

Mouse monoclonal antibodies LP34 (reactive with keratins 10, 18 and complexes of keratins 5 and 14, 6 and 16; Lane *et al.* 1985) and LE61 (reactive with keratin 18; Lane, 1982) were gifts from Dr E. B. Lane, ICRF. Mouse monoclonals to human P-cadherin and E-cadherin (NCC-CAD-299 and HECD-1, respectively; Shimoyama *et al.* 1989) were gifts from Dr S. Hirohashi, National Cancer Research Institute, Tokyo, Japan. Mouse monoclonals to the α_2 integrin subunit (5E8; Zylstra *et al.* 1986) and the α_3 integrin subunit (P1B5; Wayner and Carter, 1987) were gifts from Dr R. Bankert, Roswell Park Memorial Institute, Buffalo, NY, and Dr E. Wayner, Oncogen, Seattle, WA, respectively. Monoclonal antibodies SM-1, to smooth muscle actin, and DP2.15, to desmoplakins I and II, and FITC-conjugated second antibodies were purchased from ICN Biomedicals, High Wycombe, Bucks, UK.

Cell culture

ndk (passages 5–12) were grown in a medium composed of 3 parts DMEM, 1 part Ham's F12, supplemented with 10% FCS (Sera-Labs, Crawley Down, Sussex, England), 1.8×10^{-4} M adenine, 5 μg ml^{-1} insulin (Sigma), 0.5 μg ml^{-1} hydrocortisone (Calbiochem, Cambridge Bioscience, Cambridge, England), 10^{-10} M cholera toxin (ICN Biomedicals, High Wycombe, Bucks, UK) and 10 ng ml^{-1} EGF (a gift from Dr George-Nascimento, Chiron Corporation, Emeryville, CA) (FAD+FCS+HICE; Allen-Hoffmann and Rheinwald, 1984). Normal human epidermal keratinocytes (strain a, passages 8–10) were grown in the same medium in the presence of 3T3 clone J2 (J2–3T3) cells pretreated for 2 h with 4 μg ml^{-1} mitomycin C (Sigma; Rheinwald and Green, 1975). J2–3T3 cells were grown in DMEM containing 10% newborn calf serum. Madin Darby Canine Kidney Cells (MDCK; Madin and Darby, 1958), MG-63 human osteosarcoma cells (Billiau *et al.* 1977) and ras-3T3 NIH/2 clone D4 cells (ras-3T3; Stoker *et al.* 1987) were grown in DMEM containing 10% fetal calf serum. All cells were maintained at 37 °C in a humidified 5% CO_2 atmosphere and media were changed every 2–3 days.

Time-lapse cinemicroscopy

Cells were plated in 35 mm tissue culture dishes (Nunc) and cultured for at least 24 h before use. When used, scatter factor was added immediately prior to the start of filming. Dishes were placed in sealed chambers flushed with 5% CO_2, on a microscope stage heated to 37 °C. Photographs were taken at 2 min intervals over a 48 h period using an Olympus 1M Bowex camera. Migration speeds were calculated by measuring the migration of 25 cells over a 20 h period, using the motility analysis program 'MOTA'.

Scatter factor activity assay

Samples were analysed for scatter factor activity essentially as described by Stoker and Perryman (1985). Briefly, doubling dilutions of purified scatter factor or conditioned media in DMEM+5% FCS were prepared in 96-well tissue culture plates (Nunc). 3×10^3 MDCK cells were added per well in the same medium to bring the final volume to 0.3 ml, and the plates were incubated overnight at 37 °C. Plates were then rinsed in PBS, the cells were fixed in 3.7% formaldehyde, stained with methylene blue, and their degree of scattering assessed using a Wild dissecting microscope. Photographs were taken using a Leitz Labovert microscope. The highest dilution of sample that caused noticeable cell scattering was defined as the titre; divided by 0.3 this gives the units of scatter factor activity per ml.

Partial purification of scatter factor

Scatter factor was prepared from serum-free medium conditioned by *ras*-transformed NIH 3T3 cells (clone D4) essentially as described (Gherardi *et al.* 1989), but using cells adherent to Whatman glass 1.04 microcarrier beads (Whatman, Maidstone, Kent, UK) and a heparin–Sepharose chromatography step (Rosen *et al.* 1989). FAD+FCS+HICE medium conditioned for 72 h by ndk was filtered through a 0.22 μm pore filter (Nalgene) and concentrated fivefold by precipitation with 60% ammonium sulphate. Medium conditioned for 72 h by ras-3T3 cells was concentrated 24-fold by ultrafiltration using a PM30 membrane (Amicon). Batches of ras-3T3 scatter factor concentrated by either of these methods had the same biochemical properties (R. Furlong, unpublished observations). Concentrated medium was dialysed against 0.01 M sodium phosphate, pH 7.3, 0.15 M NaCl and then loaded onto an 8 ml heparin–Sepharose column (Pharmacia, Uppsala, Sweden) at a flow rate of 0.5 ml min^{-1}. The column was eluted with a linear gradient of NaCl (0.15 M to 2 M) and 1 ml fractions were collected and assayed for scatter factor activity. Active fractions were pooled, dialysed against 50 mM Mes, pH 6.0, 0.25 M NaCl, and loaded onto a Mono S cation exchange FPLC column (Pharmacia-LKB, Bromma, Sweden) at a flow rate of 1 ml min^{-1}, at room temperature. Bound proteins were eluted with a linear gradient of NaCl (0.25 M to 1 M); 1 ml fractions were collected and assayed for scatter factor activity.

The amount of protein in purified ndk scatter factor preparations was too low for accurate determination by spectrophotometric analysis. The specific activity of Mono S-purified ras-3T3 scatter factor was approximately 2 units ng^{-1}.

Preparation of antiserum to scatter factor

ras-3T3 cell scatter factor was purified by heparin–Sepharose and Mono S chromatography as described above and resolved on a 15% polyacrylamide gel under non-reducing conditions, using the method of Laemmli (1970). The gel was cut into 3 mm wide horizontal slices and proteins were eluted from each slice and tested for scatter factor activity as described (Gherardi *et al.* 1989). Active scatter factor was eluted from the region containing proteins of $58\times10^3 M_r$–$65\times10^3 M_r$ and was concentrated by ethanol precipitation. Approximately 15 μg of eluted, concentrated material was mixed with Freund's complete adjuvant and used for subcutaneous immunisation of a DA rat. The rat was boosted three times, at 3-week intervals, with 3–5 μg of scatter factor in Freund's incomplete adjuvant and then bled 7 days later. Subsequently, the rat was immunised at 12- to 14-week intervals and bled 7 days after each immunisation.

Gel electrophoresis and Western blotting

Samples of scatter factor were resolved on 15% polyacrylamide gels prepared and run accordingly to the method of Laemmli (1970). Samples were either run non-reduced or after reduction by treatment with 5% 2-mercaptoethanol at 100 °C for 2 min. For Western blotting, proteins were transferred to nitrocellulose (0.45 μm pore size, Schleicher and Schuell, FRG) at 1 mA cm^{-2} for 1 h in a buffer composed of 39 mM glycine, 48 mM Tris, 0.0375% SDS and 20% methanol, using a Novablot Electrophoretic Transfer kit (Pharmacia LKB).

The nitrocellulose was cut into strips 2 mm wide, blocked with PBS containing 5% BSA and incubated with rat antiserum to scatter factor diluted in PBS containing 1% BSA for 1 h. After washing, bound antibodies were visualised using immunogold-conjugated goat anti-rat IgG with silver enhancement (Janssen Life Sciences Products, Wantage, Oxon, UK) according to the manufacturer's instructions. To visualise total bound proteins

and the molecular weight markers, strips were stained with Aurodye forte (Janssen), again according to the manufacturer's instructions.

Indirect immunofluorescence

Cells grown on tissue culture plastic or on glass coverslips were fixed as follows: in 1:1 (v/v) methanol/acetone for 10 min on ice for staining with anti-keratin antibodies; in absolute methanol for 10 min on ice for staining with anti-desmoplakin antibodies; in PBS containing 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 3.7 % formaldehyde for 10 min at room temperature for staining with antibodies to cadherins, integrins or peanut lectin-binding glycoproteins; in PBS containing 3.7 % formaldehyde followed by absolute methanol for 10 min at −20 °C for staining with anti-involucrin serum; in PBS containing 3.7 % formaldehyde followed by 10 min in 50 mM Mes, pH 6.1, 5 mM $MgCl_2$, 3 mM EGTA, 100 mM KCl and 0.2 % Triton X-100 at room temperature for staining with phalloidin (O'Neill *et al.* 1990).

Cells were incubated with first antibody for 45 min at room temperature, washed extensively in PBS, incubated with the appropriate FITC-conjugated second antibody (ICN Biomedicals), washed again in PBS, mounted in Gelvatol (Monsanto Co., St. Louis, MO) and examined under epifluorescence using a Zeiss Axiophot microscope.

Results

ndk are motile cells

In culture ndk display morphological features that suggest they are motile cells. The cells grow in isolation rather than as coherent colonies, have prominent ruffled membranes and abundant cell surface microvilli, and form few desmosomes (Adams and Watt, 1988; see also Fig. 4A). We used time-lapse cinemicroscopy to investigate ndk motility in more detail. In subconfluent ndk cultures, cells moved non-directionally with a mean speed of 16 $\mu m\,h^{-1}$ (range 6–23 $\mu m\,h^{-1}$, $n=25$). Migrating cells usually had a markedly polarised morphology, with a well-spread leading lamella and a trailing tail. Some cells were non-migratory and underwent circumferential membrane ruffling. When cells contacted each other, membrane ruffling continued and they did not form stable cell–cell adhesions but tended to move apart. Cell migration decreased as the culture reached confluence, although some membrane activity remained. Since EGF is required for ndk growth (Adams and Watt, 1988) and stimulates the migration of normal keratinocytes (Barrandon and Green, 1987), we examined ndk motility in the absence of EGF. We found that the mean velocity of ndk decreased to 9.6 $\mu m\,h^{-1}$, although the range was similar to that of the control cells grown in the presence of EGF (2–32 $\mu m\,h^{-1}$, $n=25$) and the cells had a less polarised morphology than control cells (results not shown).

We also examined the effect of scatter factor on ndk motility, since this factor can increase the motility of epithelial cells. When 4.8 units ml^{-1} scatter factor was added to the cultures just before filming started, no increase in cell movement was apparent. It is possible that higher concentrations would have an effect; nevertheless, the behaviour of ndk contrasts markedly with that of MDCK, which undergo a dramatic decrease in colony cohesion and an increase in movement of individual cells in 4.8 units ml^{-1} scatter factor (Fig. 1A,B). The effects of scatter factor are not dependent on the scatter factor and the responding cells being derived from the same species (Stoker and Perryman, 1985; Stoker *et al.* 1987).

ndk produce scatter factor

To test the possibility that ndk did not respond to exogenous scatter factor because they were producing their own, we examined the activity of ndk conditioned medium in the MDCK cell scattering assay. This medium indeed caused scattering of MDCK cells (Fig. 1C), whereas medium conditioned by normal human epidermal keratinocytes was inactive (Stoker *et al.* 1987; and results not shown). The titre of separate batches of conditioned medium varied between 1:16 and 1:64. At a titre of 1:16, this corresponds to production of 50 units ml^{-1} by 10^7 cells over a 48 h period.

To discover whether the scattering activity in the medium was due to the presence of the previously characterised scatter factor, ndk conditioned medium was purified in parallel with concentrated conditioned medium of ras-3T3 D4 cells (Gherardi *et al.* 1989). The media were passed over a heparin–Sepharose column and eluted with linear salt gradients. Using either source, the peak of scatter factor activity eluted at 1.7 M NaCl (Fig. 2A,B). In each case, the active fractions were pooled, dialysed and passed down a mono S cation-exchange column and eluted with salt gradients. Again, the peak of scatter factor activity eluted at the same point on both gradients, that is, at 0.7 M NaCl (Fig. 2C,D). These results indicated that molecular species with similar properties were responsible for the MDCK cell scattering activity of both ndk and ras-3T3 conditioned media.

To extend this identification, samples of Mono S-purified ndk and ras-3T3 scatter factor were resolved on a 15 % polyacrylamide gel under reducing conditions, transferred to nitrocellulose and incubated with a rat antiserum raised against highly purified ras-3T3 scatter factor. This antiserum reacts on Western blots of ras-3T3 scatter factor with non-reduced, $62\times10^3\,M_r$ scatter factor (not shown) and, after reduction of the sample, with the $57\times10^3\,M_r$ species (Fig. 3, lane 2). An immunoreactive species of the same molecular weight was present in the ndk samples (Fig. 3, lane 5); this $57\times10^3\,M_r$ band was not detected in lanes probed with preimmune serum (Fig. 3, lanes 3 and 6).

These results show that ndk, which are human epithelial cells, secrete a protein that shares biological activity and biochemical and immunological characteristics with the previously purified scatter factor produced by fibroblastic cells.

Suramin blocks scatter factor activity

To test whether autocrine production of scatter factor was responsible for some of the unusual phenotypic features of ndk, we sought an agent that could prevent the interaction of scatter factor with cells. Suramin is a polyanionic detergent that is known to prevent the binding of various polypeptide growth factors to their receptors and also to remove growth factors from occupied receptors (Hosang, 1985; Coffey *et al.* 1987). Since there are no radioligand binding assays currently available for scatter factor, we investigated the activity of suramin in the scatter factor activity assay.

Suramin at concentrations up to and including 250 $\mu g\,ml^{-1}$ had no effect on the morphology or viability of MDCK (see Fig. 1D), but was somewhat cytotoxic at a concentration of 500 $\mu g\,ml^{-1}$. 4.8 units ml^{-1} of ras-3T3 scatter factor, or 2.5 units ml^{-1} of ndk scatter factor caused pronounced scattering of MDCK (Fig. 1A–C). However, in the presence of increasing concentrations of suramin

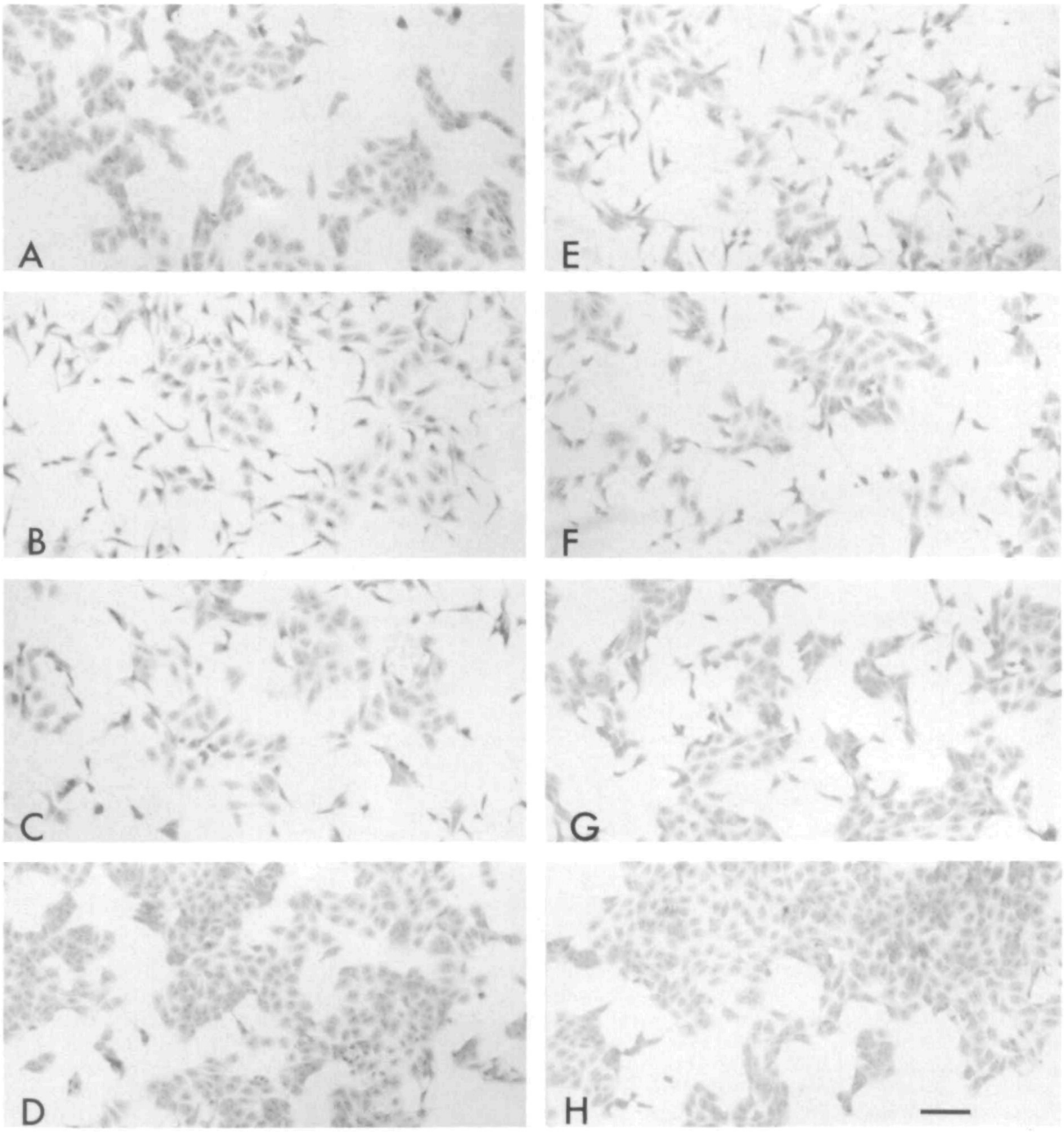

Fig. 1. Inhibition by suramin of scatter factor activity. MDCK cells were plated overnight under various conditions, then washed, fixed and stained. (A) Untreated MDCK; (B) cells treated with 4.8 units ml^{-1} of ras-3T3 scatter factor; (C) cells treated with 2.5 units ml^{-1} of ndk scatter factor; (D) cells treated with 250 $\mu g\,ml^{-1}$ suramin; (E–H) cells treated with 2.5 units ml^{-1} of ndk scatter factor and suramin at: (E) 50 $\mu g\,ml^{-1}$; (F) 100 $\mu g\,ml^{-1}$; (G) 175 $\mu g\,ml^{-1}$; (H) 250 $\mu g\,ml^{-1}$. Bar, 100 μm.

(50–250 $\mu g\,ml^{-1}$) the cells were progressively less well scattered by scatter factor from both sources (Fig. 1E–H and results not shown). Thus, in the presence of 2.5 units ml^{-1} of ndk scatter factor and 250 $\mu g\,ml^{-1}$ suramin, MDCK morphology was almost indistinguishable from that of untreated control MDCK (Fig. 1A,H). These results suggest that suramin prevents the interaction of scatter factor with its cellular binding sites.

Effect of suramin on ndk morphology

We next examined the response of ndk to suramin treatment. ndk were plated in the presence of a range of concentrations of suramin (50 $\mu g\,ml^{-1}$–1 $mg\,ml^{-1}$) and their morphology was examined 24 h later. Cells treated with 50 $\mu g\,ml^{-1}$ or 100 $\mu g\,ml^{-1}$ suramin were indistinguishable from the controls (not shown). Suramin concentrations of 250 $\mu g\,ml^{-1}$ and above caused a pronounced change in cell morphology. The cells formed coherent groups, but did not stratify. Cells in the groups had less clearly ruffled membranes than control cells and elongated cells were observed apparently associating with or dissociating from the groups (Fig. 4B). Some isolated, single cells always remained; these tended to be rounded and less well spread than control ndk and rarely displayed the polarised, motile morphology (Fig. 4B,D).

Cells fed with medium containing suramin for 8 days

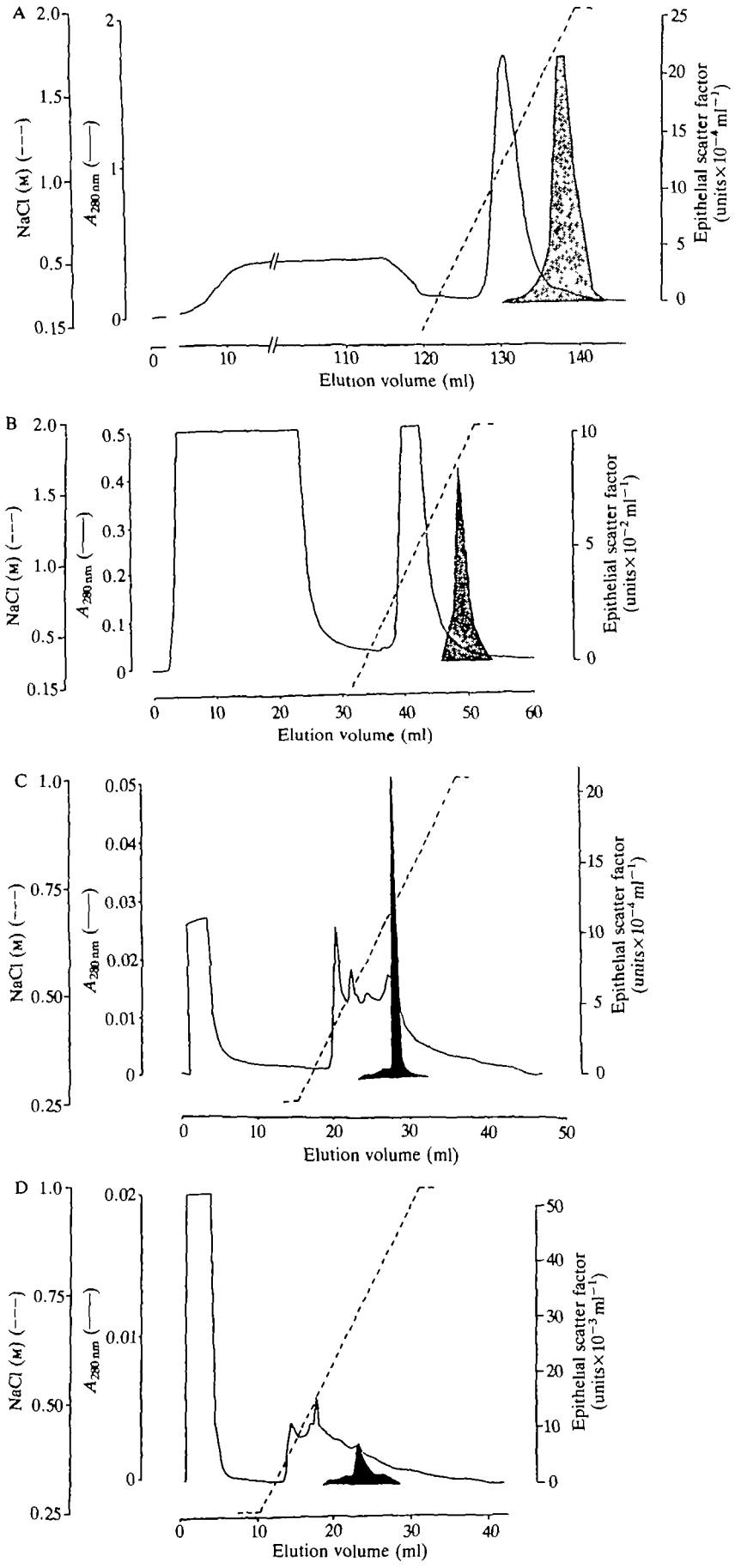

Fig. 2. Purification of scatter factor from ndk or ras-3T3 conditioned media. Conditioned media of ras-3T3 (A and C) or ndk (B and D) were passed over heparin–Sepharose columns (A,B) and eluted as described in the Materials and methods. Active fractions (indicated by the hatched area) were pooled and passed over Mono S cation-exchange columns (C,D; active fractions indicated by black areas).

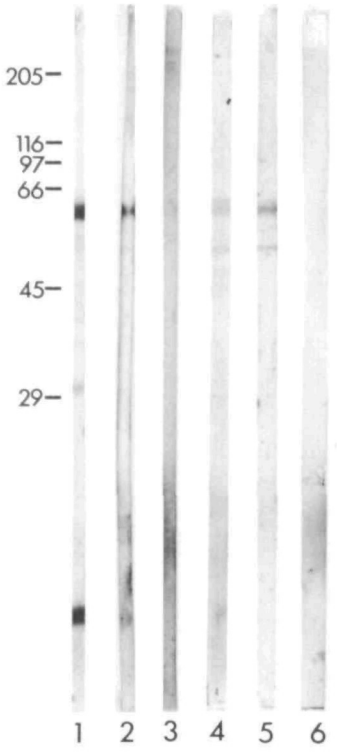

Fig. 3. Western blot of scatter factor purified from ras-3T3 cells or ndk. Scatter factor was purified from ras-3T3 (lanes 1–3) or ndk (lanes 4–6) conditioned media by passage over heparin–Sepharose and Mono S cation-exchange columns. Reduced samples were resolved on a 15% polyacrylamide gel and transferred to nitrocellulose. Lanes 1 and 4, total protein stained with Aurodye forte; lanes 2 and 5, blot probed with anti-scatter factor serum; lanes 3 and 6, blot probed with preimmune serum. ras-3T3 scatter factor activity was loaded in 100-fold excess of ndk scatter factor activity. Molecular weight standards are given ($\times 10^{-3}$).

continued to show the morphological alterations. Cells treated with up to 750 $\mu g\,ml^{-1}$ suramin continued to grow and divide, although suramin concentrations of 300 $\mu g\,ml^{-1}$ or more caused them to grow more slowly than the controls. After several days, cytotoxicity was apparent in cells treated with 750 $\mu g\,ml^{-1}$ or more of suramin (data not shown).

To find out if the effect of suramin was reversible, cells were grown in 500 $\mu g\,ml^{-1}$ suramin for 5 days and then trypsinised, washed and replated in two dishes. One dish was refed with 500 $\mu g\,ml^{-1}$ suramin and the other left untreated. 24 h later, the untreated cells were morphologically indistinguishable from control ndk, and grew normally thereafter (Fig. 4C). In contrast, cells replated in the presence of suramin remained in groups (Fig. 4D). Taken together, these experiments indicate that the effects of suramin on ndk are concentration-dependent, reversible and, except at high concentrations, nontoxic.

We also examined the effect of suramin on normal human epidermal keratinocytes and fibroblastic cell lines. Keratinocytes treated for 1–3 days with suramin at concentrations up to 1.5 $mg\,ml^{-1}$ showed no alteration in morphology or growth rate. Treatment of J2–3T3 fibroblasts or MG-63 osteosarcoma cells, both of which produce scatter factor (Stoker *et al.* 1987; J.C.A., unpublished observation), with suramin did not cause any alterations in cell morphology. This is consistent with the observation that fibroblasts do not respond to scatter factor, whether or not they produce it themselves (Stoker and Perryman, 1985; Stoker *et al.* 1987; Stoker, 1989).

The effect of suramin on ndk morphology was examined in more detail by using time-lapse cinemicroscopy. Cells pretreated with 500 $\mu g\,ml^{-1}$ suramin for 24 h were filmed for a 48 h period. These experiments showed that the cell groups moved slowly over the culture substratum. Sometimes groups pulled apart as cells at opposite ends started to move outwards. When this happened, cells in the region of the split assumed a highly elongated morphology, as seen in the fixed preparations (see Fig. 4B).

We attempted to overcome the increased coherence of the cells by simultaneously treating them with 400 $\mu g\,ml^{-1}$ suramin and 4 units ml^{-1} ras-3T3 or ndk scatter factor; however, this did not cause reversal to a normal ndk morphology (data not shown). We were unable to test the effect of the antiserum to scatter factor on ndk morphology, since insufficient quantities were available.

Effect of suramin on the expression of involucrin, adhesion molecules and cytoskeletal components

In the presence of suramin, ndk became more cohesive and the cell groups bore some resemblance to colonies of normal epidermal keratinocytes. We therefore used immunofluorescence to examine whether suramin affected the distribution of the cytoskeleton and cell adhesion molecules in ndk, or induced expression of keratinocyte terminal differentiation markers. For these experiments, cells were treated with 400 $\mu g\,ml^{-1}$ suramin and grown for 3–5 days on glass coverslips. Suramin treatment of ndk did not induce expression of involucrin, a major cytoplasmic precursor protein of the cornified envelope, and therefore a marker of terminal differentiation in normal keratinocytes (results not shown; Watt, 1989). Suramin-treated ndk, like the control cells, stained with LP34, an antibody that recognises keratins expressed by simple and stratified epithelia, and were not stained by LE61, an antibody reactive with a simple epithelial keratin (data not shown; see also Adams and Watt, 1988). Suramin did not increase the alignment of keratin filaments at cell–cell boundaries and did not increase desmosome formation (results not shown).

Suramin did cause a redistribution of the actin cytoskeleton. In control ndk, patchy actin staining was observed in areas of membrane ruffling and in spread portions of the cytoplasm. Stress fibres were present in the trailing tail of motile cells, as long fibres that spanned the length of certain cells, or in a radial distribution in spread non-polarised cells (Fig. 5A). In suramin-treated cells, stress fibres were also present, but, in addition, there were short fibres at cell–cell junctions (Fig. 5B). Suramin treatment did not induce the expression of smooth muscle actin (not shown).

Integrins and cadherins are cell adhesion molecules that interact with the actin cytoskeleton. Suramin did not increase the very low level of E- and P-cadherin staining observed in ndk, but did have a marked effect on integrins. In ndk, the $\alpha_2\beta_1$ and $\alpha_3\beta_1$ integrins have quite distinct distributions: $\alpha_2\beta_1$ is concentrated in areas of ruffled membrane, and $\alpha_3\beta_1$ is present on cell surface microvilli (Adams and Watt, unpublished; and Fig. 5C and E). Suramin treatment caused a partial redistribution of both integrins to cell–cell contact areas (Fig. 5C–F). Other integrins ($\alpha_1\beta_1$, $\alpha_5\beta_1$ and $\alpha_6\beta_4$) also appeared to be redistributed to cell–cell contact areas (data not shown). This did not reflect a general redistribution of cell surface glycoproteins, because the peanut lectin-binding glycoproteins generally remained associated with microvilli distributed over the entire upper surface of the cells (Adams and Watt, 1988; Fig. 5G,H).

Discussion

In this paper we have shown that ndk, a strain of epidermal keratinocytes, produce a factor identified as

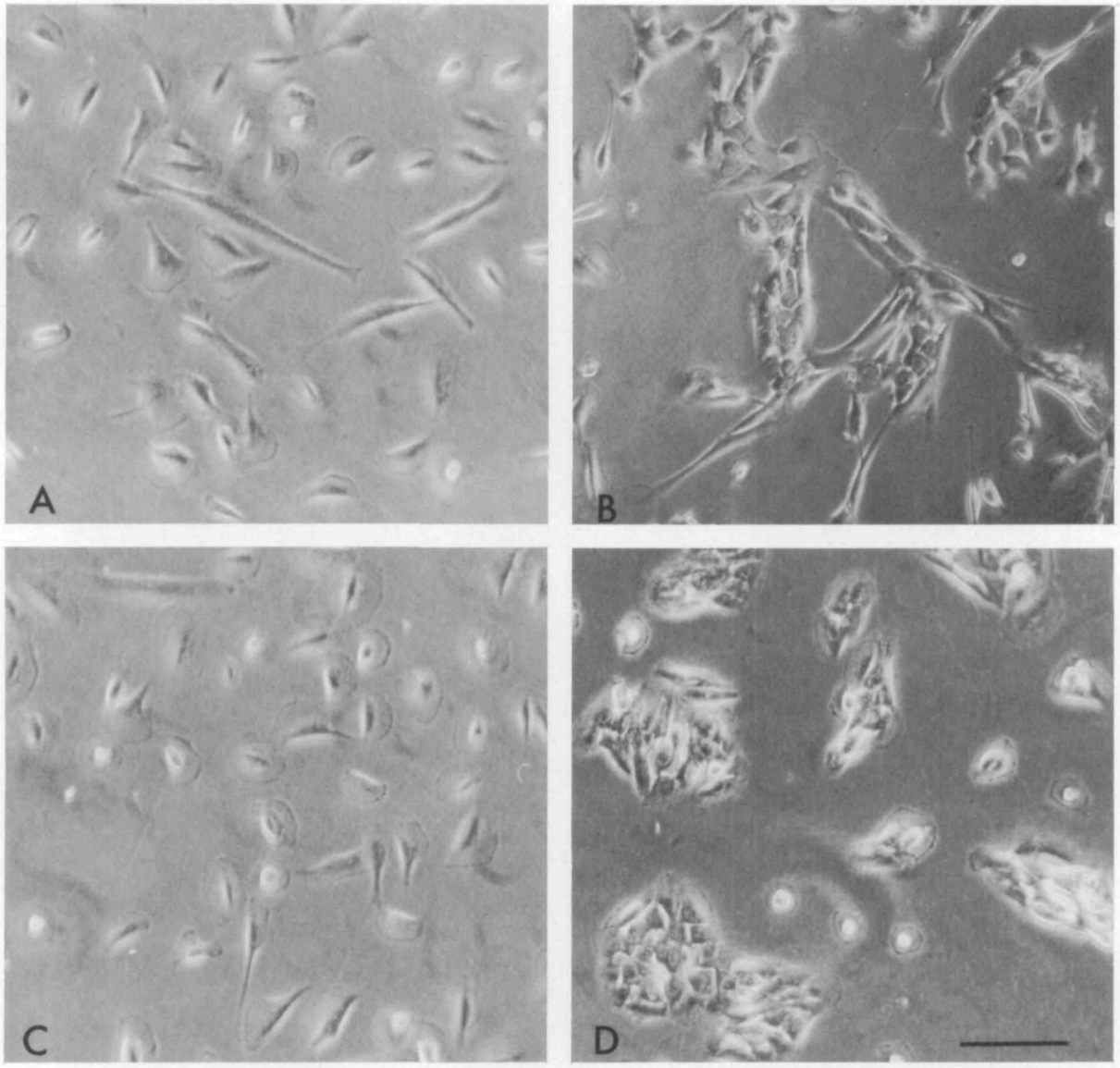

Fig. 4. Effect of suramin on ndk morphology. (A) Control ndk culture. (B) Cells plated for 24 h in the presence of 500 $\mu g\,ml^{-1}$ suramin. (C) Cells treated with 500 $\mu g\,ml^{-1}$ suramin for 5 days, 24 h after washing and replating without suramin. (D) Cells treated with 500 $\mu g\,ml^{-1}$ suramin for 5 days, 24 h after replating in the presence of 500 $\mu g\,ml^{-1}$ suramin. Bar, 50 μm.

scatter factor on the basis of three criteria. First, it has scatter factor activity in the standard MDCK bioassay; second, it co-purifies with fibroblast scatter factor in a partial purification protocol; and third, it is reactive with an antiserum raised against highly purified scatter factor. The production of scatter factor by ndk was low in comparison to certain fibroblastic cell lines, which may reach a titre of 1690 units ml^{-1}, but is nevertheless remarkable, because the conditioned media of other epithelial cell types contain no detectable activity (Stoker *et al.* 1987). Thus, these results are of interest because they provide an exception to the general observation that mesenchymal cells produce scatter factor whereas epithelial cells respond (Stoker *et al.* 1987).

In contrast to normal keratinocytes (Sun and Green, 1976; Magee *et al.* 1987; Stoker *et al.* 1987) ndk move as individual cells under standard culture conditions and do not increase in motility in the presence of exogenous scatter factor. This could indicate either that they lack scatter factor receptors, or that the receptors are already occupied by the scatter factor that the ndk themselves produce. The latter appears to be a likely explanation, because suramin, an agent that reverses the activity of murine scatter factor in the standard bioassay, caused ndk to become less motile and to form coherent groups of cells.

How suramin abrogates scatter factor activity is unclear. Suramin is known to bind to various polypeptide growth factors and thus could prevent the interaction of scatter factor with its putative cell surface receptors; it may also dissociate receptor-bound scatter factor by this means (Hosang, 1985; Coffey *et al.* 1987). Since scatter factor binds to heparin (Rosen *et al.* 1989), it is also possible that it binds to cell-surface or extracellular matrix-associated heparan sulphate proteoglycans; it is not known if this type of interaction could be blocked by suramin. Suramin can also act intracellularly, where it inhibits various enzymes, including protein kinase C (Hawking, 1978; Hensey *et al.* 1989; Mahoney *et al.* 1990).

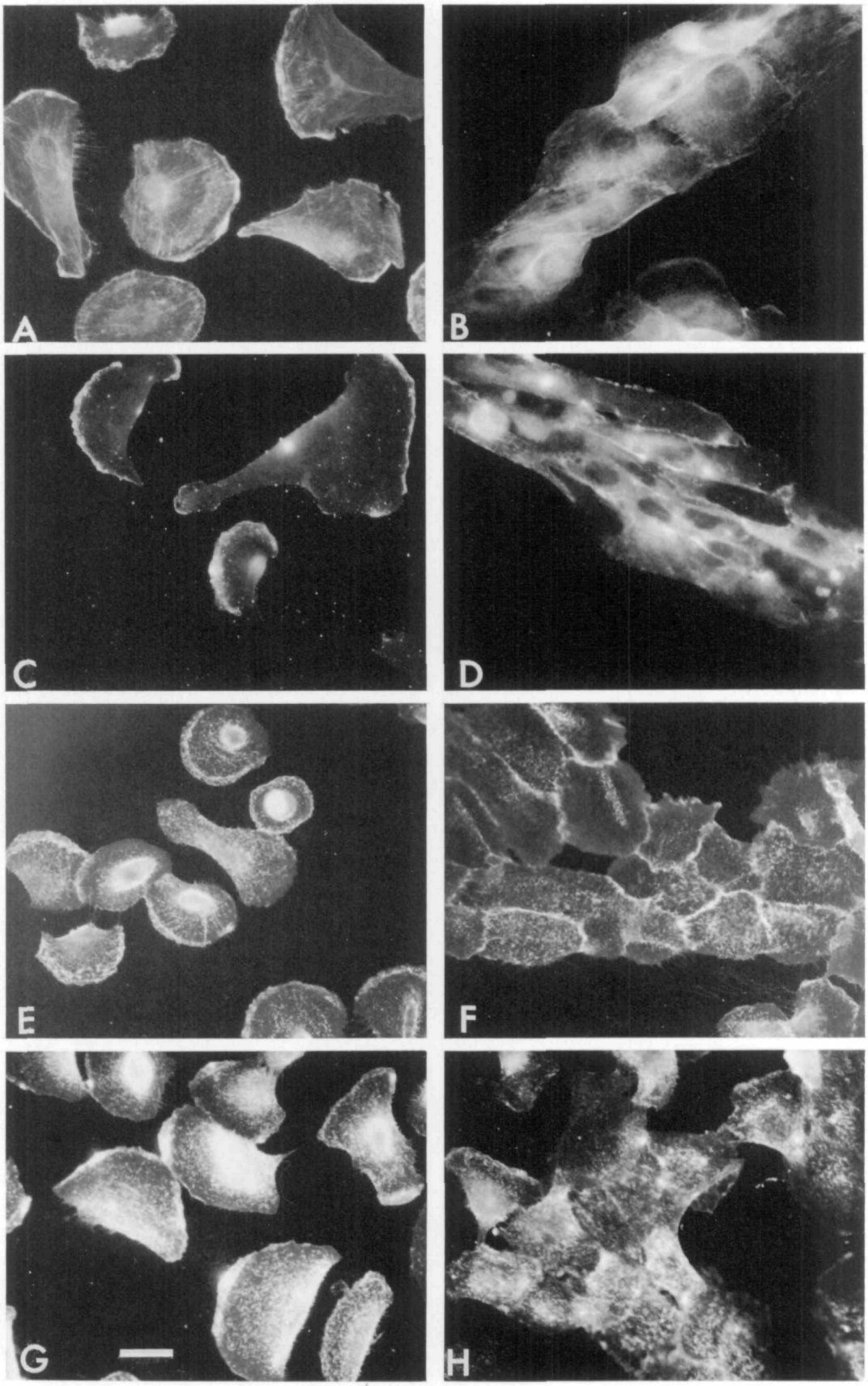

Fig. 5. Effect of suramin on the distribution of actin and cell-surface proteins in ndk. Control ndk (A, C, E and G) or ndk grown for 3 days in the presence of 400 $\mu g\,ml^{-1}$ suramin (B, D, F and H) were stained with rhodamine–phalloidin (A,B) or antibodies to the α_2 integrin subunit (C,D), the α_3 integrin subunit (E,F) or with anti-PNA-gp serum (G,H). Bar, 10 μm.

There is some suggestion that protein kinase C may be involved in the signal transduction pathways that mediate cellular responses to scatter factor (Rosen *et al.* 1990). To investigate this phenomenon further, more information on the nature of the interaction(s) of scatter factor with cells is required and suramin will be useful for these experiments.

Since we could not reverse the morphological alteration caused by suramin by addition of exogenous scatter factor, it is unlikely that scatter factor alone is responsible for the migratory phenotype of ndk. Our time-lapse experiments suggest a role for EGF in motility and suramin is known to prevent the interaction of EGF with its receptors (Coffey *et al.* 1987). Other polypeptides present in fetal calf serum or produced by the cells may also be bound by suramin. In support of this conclusion, we could not reverse the patching of ndk cells by the simultaneous addition of exogenous scatter factor and EGF (data not shown).

We found that integrins were redistributed after suramin treatment. In ndk, as in normal keratinocytes, β_1 integrins are involved in initial adhesion to extracellular matrix glycoproteins, although after several days in culture, individual integrins have different distributions and are not restricted to the basal surface of the cells (see Fig. 5C and E; Adams and Watt, unpublished data). After suramin treatment, the $\alpha_2\beta_1$ and $\alpha_3\beta_1$ integrins were both relocated to cell–cell contact areas, a distribution similar to that seen in keratinocytes. On the basis of this localisation and of the ability of anti-β_1 subunit antibodies to block cell–cell adhesion in culture, integrins have been proposed to have a role in cell–cell adhesion in epithelial cells (Kaufmann *et al.* 1989; Larjava *et al.* 1990; Carter *et al.* 1990). Whether integrins have any function in cell–cell adhesion in suramin-treated ndk remains to be determined.

Suramin has been reported to induce the differentiation of adenocarcinoma and neuroblastoma cell lines (Fantini *et al.* 1989; Hensey *et al.* 1989). However, even though ndk assumed a more epithelial-like morphology in the presence of suramin, there was no evidence for either stratification or terminal differentiation in the colonies. Other features of normal keratinocytes were also lacking: desmosomes and cadherins did not accumulate at sites of cell–cell apposition and the actin microfilament network did not become concentrated in bundles parallel to the cell margins (Watt *et al.* 1984; Magee *et al.* 1987; Shimoyama *et al.* 1989). We conclude that increased cell–cell contact is not a sufficient stimulus to induce terminal differentiation and stratification of ndk.

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