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Human keratinocytes migrate to the negative pole in direct current electric fields comparable to those measured in mammalian wounds

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SUMMARY

Previous measurements of the lateral electric fields near skin wounds in guinea pigs have detected DC fields between 100-200 mV/mm near the edge of the wound. We have studied the translocation response of motile primary human keratinocytes migrating on a collagen substrate while exposed to similar physiological DC electric fields. We find that keratinocytes migrate randomly on collagen in fields of 5 mV/mm or less, but in larger fields they migrate towards the negative pole of the field, exhibiting galvanotaxis. Since these cells have an average cell length of 50 μm, this implies that they are able to detect a voltage gradient as low as 0.5 mV along their length. This cath-odally-directed movement exhibits increased directedness with increasing field strengths between 10 and 100 mV/mm. We observe a maximally directed response at 100 mV/mm with half of the cells responding to the field within 14 minutes. The average speed of migration tended to be greater in fields above 50 mV/mm than in smaller fields. We conclude that human keratinocytes migrate towards the negative pole in DC electric fields that are of the same magnitude as measured in vivo near wounds in mammalian skin.

Key words: Galvanotaxis, Keratinocyte, Wound healing, Electric field

INTRODUCTION

Motile cells use many different signals in their environment to guide their migration. Among the most thoroughly studied of these types of directed migration are chemotaxis, haptotaxis, and contact guidance. The guidance of motility by endogenous electric fields, known as galvanotaxis, is not as well characterized as these other responses, but has now been documented in more than 14 cell types (Nuccitelli, 1988; Robinson, 1985) and may play an important role in those cellular responses where local electric fields are present. Mammalian granulocytes (Gruler, 1993), bone cells (Ferrier et al., 1986), amphibian (Stump and Robinson, 1983) and avian (Nuccitelli and Smart, 1989) neural crest cells, avian embryonic (Erickson and Nuccitelli, 1984) and murine (Brown and Loew, 1994) fibroblasts, as well as fish and amphibian epithelial cells (Cooper and Schliwa, 1985; Luther et al., 1983) respond to imposed DC electric fields by migrating towards the cathode or the anode in vitro. Here we investigate the response of human skin cells or keratinocytes to physiological DC electric fields. We use the term physiological because we have used DC fields of a similar magnitude to those endogenous DC fields measured near wounds in mammalian skin (Barker et al., 1982). To completely reepithelialize a wound, keratinocytes must migrate in a directed, rather than random, fashion and the signals to guide directed motility in skin wounds are largely unknown. Here we propose that the electric field generated by the wound currents provides the keratinocytes with one of the earliest cues necessary for this directed migration.

For more than a century, we have known that electrical currents in the order of one μA flow out of wounds in human skin (DuBois-Reymond, 1843), and modern studies have detected 10-100 $\mu A/cm^2$ leaving epithelial wounds (Illingworth and Barker, 1980; Chiang et al., 1992). A transepidermal voltage gradient, created by the sodium ion pumps of the epithelium, acts as the driving force for this electrical current which flows through the low resistance pathway of the wound. Transepithelial potentials (TEP) between 20 and 50 mV, inside positive, have been recorded from regions of intact mammalian skin and comparable TEP values have been obtained at numerous locations on the human body (Barker et al., 1982).

This study provides evidence that the migration of human keratinocytes is guided by electric fields of the same magnitude found in mammalian wounds. This result suggests that galvanotaxis may be one of the physiological mechanisms involved in wound healing.

MATERIALS AND METHODS

Cell culture

The human keratinocytes used in this study were derived from a single neonatal donor's foreskin epidermis and cultivated, using the method described by Rheinwald and Green (1975), in Dulbecco's modified Eagle's medium (DMEM; Flow Laboratories, McLean, VA) supplemented with 10% (v/v) fetal calf serum (FCS; Hyclone Laboratories, Inc., Logan, UT), 0.4 μg/ml hydrocortisone, and 10 ng/ml EGF on a feeder layer of 3T3 cells. Cells were stored in liquid nitrogen (as

passage two) and subcultured as needed in keratinocyte medium 154 (Cascade Biologics, Inc., Portland, OR), supplemented with bovine pituitary extract, insulin, hydrocortisone, transferrin and human epidermal growth factor as previously described (Pittelkow and Scott, 1986) and 100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin at 37°C in a humidified atmosphere of 5% CO_2. For each experiment, cells were removed from liquid nitrogen and grown for 4-8 days in the incubator before removing them from the culture plate by trypsinization and culturing on our electric field chambers for two hours. Only such 3rd passage cells were used in the experiments and all the data in this paper were collected from cells derived from a single donor. These results were confirmed, however, with cells taken from three other donors.

Glass coverslips (25 mm × 6 mm) were washed in 15% nitric acid, autoclaved, then incubated for 12 hours at 37°C with 2 ml of a 2% collagen-I solution (Celtrix Pharmaceuticals, Inc., Santa Clara, CA) in CMF-PBS (final concentration of 62 μg/ml). Collagen I was selected to best approximate in vivo conditions where epidermal cells must migrate on dermal collagen (primarily type I). Two hours prior to the experiment, the coverslips were air-dried and plated with keratinocytes suspended in 2 ml of 1.8 mM Ca^{2+} at a density of 7.4×10^3 cells/cm^2. Non-adherent cells were washed off the coverslips with 1 ml keratinocyte medium supplemented as above, containing 1.8 mM Ca^{2+}, 10 mM Hepes buffer, pH 7.4. The coverslips were then placed in the chambers described below. Only buffered, supplemented medium, pH 7.4, containing 1.8 mM Ca^{2+} was used throughout the experiments. The population of cells was monitored by immunoperoxidase staining for keratin and vimentin, and consisted of >95% keratin-positive cells.

DC electric field application

Direct electric fields were applied to keratinocytes using an apparatus designed and used in previous galvanotaxis studies (Erickson and Nuccitelli, 1984). The apparatus consists of a plexiglass chamber with a bottom composed of a 45 mm × 50 mm piece of no. 1.5 coverslip glass to allow for direct observation of cells with an inverted compound microscope. The cells were plated onto a second coverslip that fits in the chamber between two slightly thicker pieces of glass, then a top coverslip was placed over the cells leaving a small space (less than 150 μm high) through which the current could flow. Because this resulted in a very small cross-sectional area for current flow, electrical resistance was increased, thereby minimizing the current (and ohmic heating) necessary to generate a given voltage gradient. Chambers were sealed with tape and silicone high vacuum grease. Experimental medium was added to one well of the chamber until flow was detected in the opposite well, indicating a continuous path for fluid flow over the cells. The voltage across each chamber was checked periodically by inserting Ag-AgCl electrodes into the wells on either end, and the current passing over the cells was monitored continuously during the experiment with an ammeter in series. The direct current was carried to the cells via 6 cm long agar bridges inserted at each end of the chamber which separated the cells from the electrodes and any electrode byproducts.

Safeguards to prevent excessive heat exposure of cells included minimizing the current flow which in turn minimizes joule heating of the chamber, and placing the cells on a metal plate through which water driven by a circulating water bath adjusted to 37°C flowed. The temperature of the medium surrounding the cells was checked before and after each experiment by inserting a miniature temperature probe into the wells of each chamber.

Filming procedure and data analysis

Cells were observed with phase contrast optics on an inverted microscope at ×250 magnification and their movements were recorded using a video camera and time-lapse video recorder. The region of the coverslip selected for recording typically contained 15 cells maximally dispersed to minimize contact between cells. Greater than 90% of cells remained isolated or acquired transient intercellular contacts for less than a 30 minute interval during each experiment. Those that paired or grouped (≥3) for a period longer than 30 minutes were analyzed separately. Experiments were conducted for 2.5 hours in a continuous DC electric field. Total cell displacement (net cellular translocation) was measured from the starting position for each cell to its location at the end of the 2.5 hour period. A point identifying the center of mass for each cell was recorded on plastic transparencies taped to the video screen every 15 minutes. A digitizing pad (Houston Instruments) and a computer were used to analyze the cell displacement. To quantitate the directedness of the average cellular translocation, the angle at which each cell moved with respect to the imposed electric field direction was used. The cosine of this angle would give a value of +1 for a cell that migrated directly toward the negative pole, zero for a cell migrating exactly perpendicular to the direction of the electric field, and −1 for a cell that migrated directly toward the positive pole of the field. In order to quantitate the average directedness of movement, the cosine values were averaged using the formula, $\langle\cos\phi\rangle = \Sigma_i \cos\phi_i/N$, where Σ_i= summation of cosine values obtained from individual cells from 5-10 experiments at a given electric field strength, ϕ is the angle between the field axis and the net cellular translocation direction, and N is the total number of cells contained in all of the experiments at that field strength. The net distance traveled after the first two hour period was used to derive the *net translocation velocity*. In contrast, the *track migration velocity* was calculated by measuring the distance traveled by the cell at 15 minute intervals along its migratory pathway, and describes the speed of locomotion along the course of migration during the first 2 hour period. If a cell changes direction several times, the net translocation velocity will be much smaller than the track velocity. A comparison of the two velocities (net translocation velocity/track velocity) gives the *migration index*, which describes the migration pattern of the cell. A migration index of one indicates that a linear pathway was taken from origin to endpoint, whereas a value much less than one describes a more tortuous pattern of migration.

Experiments in which the direction of the electric field was reversed were conducted in the same manner as described above, except following one hour of field exposure, the electrodes of the power supply were reversed in polarity without disturbing the video recording process.

Charge gradient control experiments

To determine whether cells were responding directly to the electric fields or to a field-induced concentration gradient of some charged factor(s) produced by the cells, a flow of medium (0.77 ml/second) perpendicular to the direction of the current was imposed for a two hour period. The average cosine of the cellular translocation distribution was then assessed. This flow rate was quite strong and could be observed in our time-lapse records to completely change the normal flow in the chamber from a pattern that followed the electric field lines to one that moved perpendicular to the field lines, and should therefore radically alter any charged molecule distributions.

RESULTS

We have studied the motility and translocation of 715 human keratinocytes migrating on a collagen substrate exposed to physiological electric fields using time-lapse video microscopy. Using field strengths comparable in magnitude to those measured near wounds in mammalian skin (Barker et al., 1982), we observed that the keratinocytes migrated randomly in fields lower than about 10 mV/mm and migrated toward the negative pole for fields between 10 and 400 mV/mm with a maximal response occurring for fields greater than or equal to 100 mV/mm. Photomicrographs taken at 0, 60, and 120

minutes after exposure to a 100 mV/mm field demonstrate the migratory path toward the negative pole (Fig. 1).

Directional migration

Isolated human keratinocytes in culture assume a variety of shapes, such as circular (ca. 40 μm diameter), polygonal, crescenteric (30 × 60 μm), and elongated (20 × 75 μm) shapes (examples of these can be seen in Fig. 1). The majority of motile cells have lamellipodial extensions along the convex side of the cell (crescenteric shape) or at two or three points along the periphery of the cell (polygonal). The circular cells are typically stationary, with thin lamellae extending around the entire cell. Often these cells spread to a size of 75 × 75 μm.

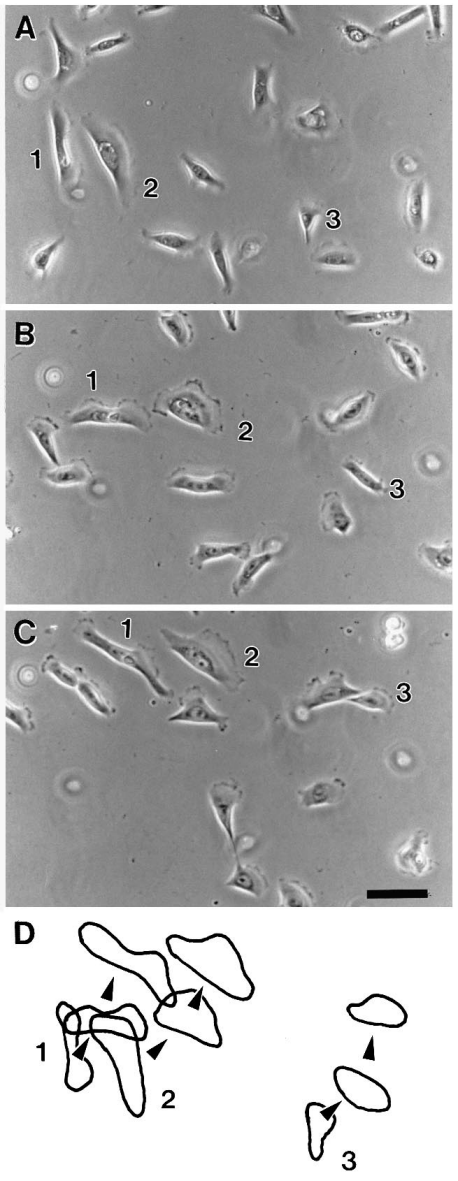

Fig. 1. Time-lapse photography showing displacement of human keratinocytes in a 100 mV/mm electric field. The cathode is at the top of each photograph. (A) Photomicrograph of cells at time 0 with three of the cells labeled with numbers. (B) Identical field of cells as in 'A' taken 60 minutes later. (C) Identical field as in 'A' taken 120 minutes later. (D) Outlines of labeled cells in A-C to indicate their migration patterns. Bar, 50 μm.

An efficient method for presenting the observed cellular migration data is in a scatter plot with the final location of each cell after migrating for 2.5 hours in a given field strength represented as one point, and with each cell's starting position placed at the origin (Fig. 2). We have quantitated the directedness of the galvanotactic response by calculating the average cosine of the angles of net translocation with respect to the electric field direction for all of the keratinocytes studied. Since a given cos ϕ will have a value of plus one if the cell moved directly towards the negative pole of the field and a value of minus one if the cell moved directly towards the positive pole, random movement of many cells would average out to zero if there is no biasing influence. We detected no significant asymmetry in the translocation distribution in a 5 mV/mm field ($<\cos \phi> = 0.03 \pm 0.06$), but in a 10 mV/mm field the cells migrated asymmetrically, favoring the negative pole ($<\cos \phi> = 0.26 \pm 0.08$). The average cosine increased with electric field strength between 10 and 100 mV/mm and leveled off in fields larger than 100 mV/mm (Fig. 2).

The distribution of the cellular translocation can also be presented as a histogram of the number of cells completing their 2.5 hour journey within a given 10° sector after starting from the origin (Fig. 3). When the number of cells in each sector is roughly the same, a random migration pattern is indicated as is the case for 0, 1 and 5 mV/mm. For 10 mV/mm and above, the histograms clearly indicate an asymmetrical distribution with more cells falling in the sectors near 0° than elsewhere.

For those electric field strengths capable of inducing galvanotaxis, we assessed the time course of the galvanotactic response (Fig. 4). Within the first 15 minutes of field exposure, the average cosines of the cellular translocation distributions indicated significant cathodal migration for all these groups. Electric field strengths beyond 100 mV/mm do not significantly increase the final average cosine of the cathodal migratory response, however, the cells respond more rapidly at these higher field strengths. By the end of one hour of field exposure, the average translocation distribution for each field strength was similar in fields of 100 mV/mm and above.

Cross-flow control

In order to determine if the cells were responding to the electric field itself rather than to a field-induced concentration gradient of some charged molecules in the medium, experiments in the presence of a continuous cross-flow of medium (0.8 ml/second) perpendicular to the direction of the electrical current were performed while exposing cells to a field of 100 mV/mm. The resulting average cosine of the cellular translocation distribution ($<\cos \phi> = 0.81 \pm 0.05$; n=56) was not significantly different from that observed in cells without crossflow, suggesting that the directed cell migration is a response to the field itself rather than being due to cellular chemotaxis toward a concentration gradient of charged molecules established by the field.

Translocation and velocity

The net cellular displacements after 2.5 hours in the 100 and 400 mV/mm electric fields are significantly greater than we observed when cells were exposed to lower field strengths (Table 1). The average cellular translocation was also assessed in 15 minute intervals over the initial two hour period to

provide an ongoing measure of movement (Fig. 5). As illustrated by the slope of each line, the change in net cell displacement over time was relatively constant within each group and provides the track velocity. By comparing this incremental or track velocity with the net translocation velocity, one obtains a measure of the number of direction changes in the cellular migration path. The migration index is the ratio of these two velocities and indicates that cells take a straighter path towards the negative pole as the field strength increases (Table 1).

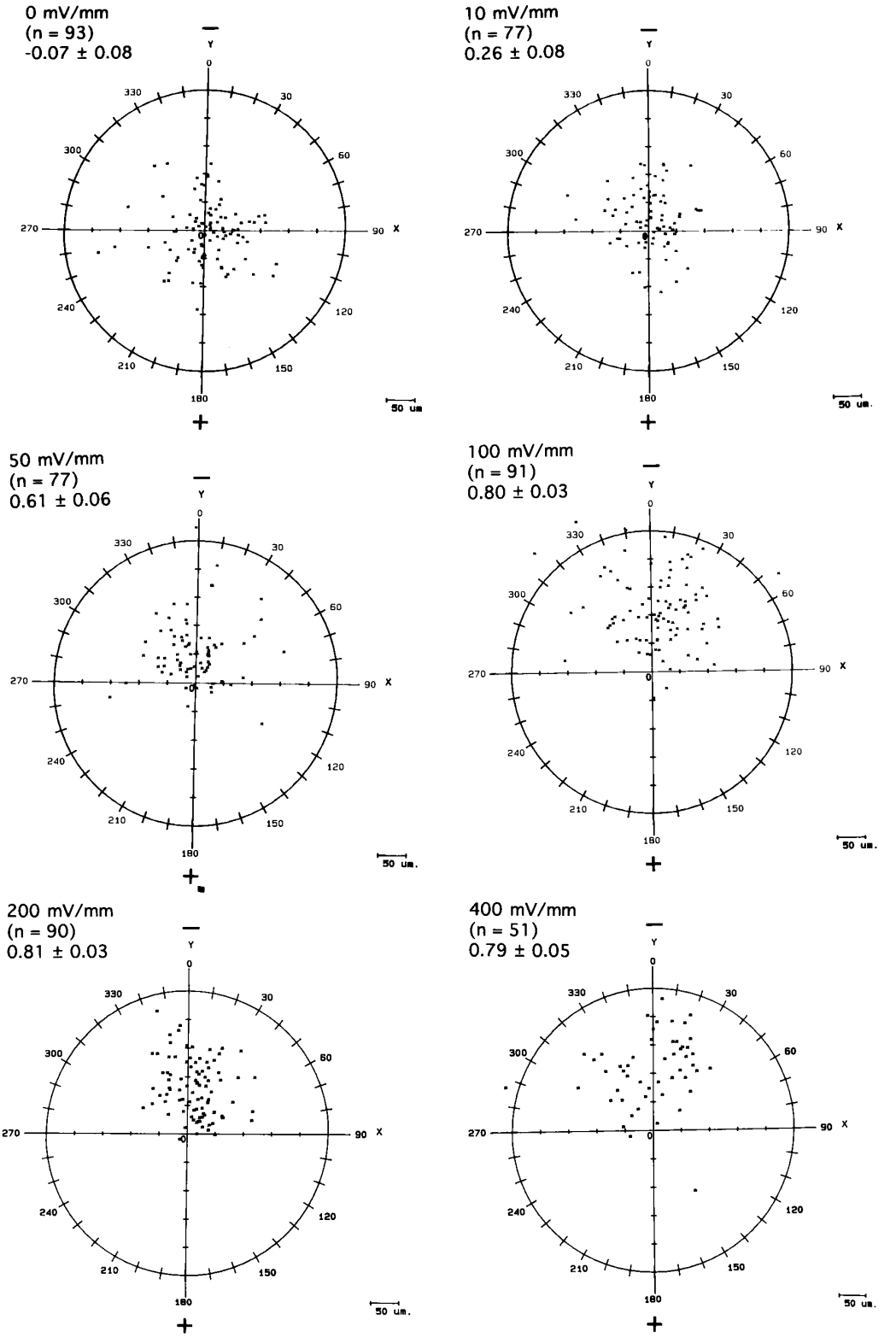

Fig. 2. The net translocation of human keratinocytes during 2.5 hours in the indicated electric field strength. Migration paths were determined by video monitor tracings. Each cell's position at t=0 minutes is represented by the origin (0,0), with the final position of each cell at t=150 minutes plotted as a single point on the graph. The radius of each circle represents 250 μm of translocation distance, and the average cell length is 50 μm. The applied electric field strength is indicated in mV/mm. n, the total number of cells studied at a given field strength. The average cosine of the distribution plus or minus the standard error of the mean is indicated in the upper left corner of each scatter plot.

Field jump (reversal) experiment

In order to better characterize the response time of human keratinocytes to electric field application, we conducted a field jump experiment in which the polarity of the electric field was reversed after the cells had been exposed to the field for one hour. By then measuring the time required for the cells to begin migrating in the opposite direction, toward the new negative pole, the time at which half of the cells had reversed their direction of migration was determined. The response half-time of human keratinocytes to imposed electric fields is the same for both the initial exposure to an electric field (14±1.1 minutes) and for a field reversal after the initial 60 minutes of field application (14±1.0 minutes) (Fig. 6). In Fig. 6 the incremental average cos ϕ is plotted rather than the net average cos ϕ as was plotted in Fig. 4. For the incremental cos ϕ, the initial position of the migration vector is the cellular location 15 minutes prior to each time point rather than that at time 0. This allows us to visualize incremental reversals in the migration direction as a change in sign of the average cos ϕ.

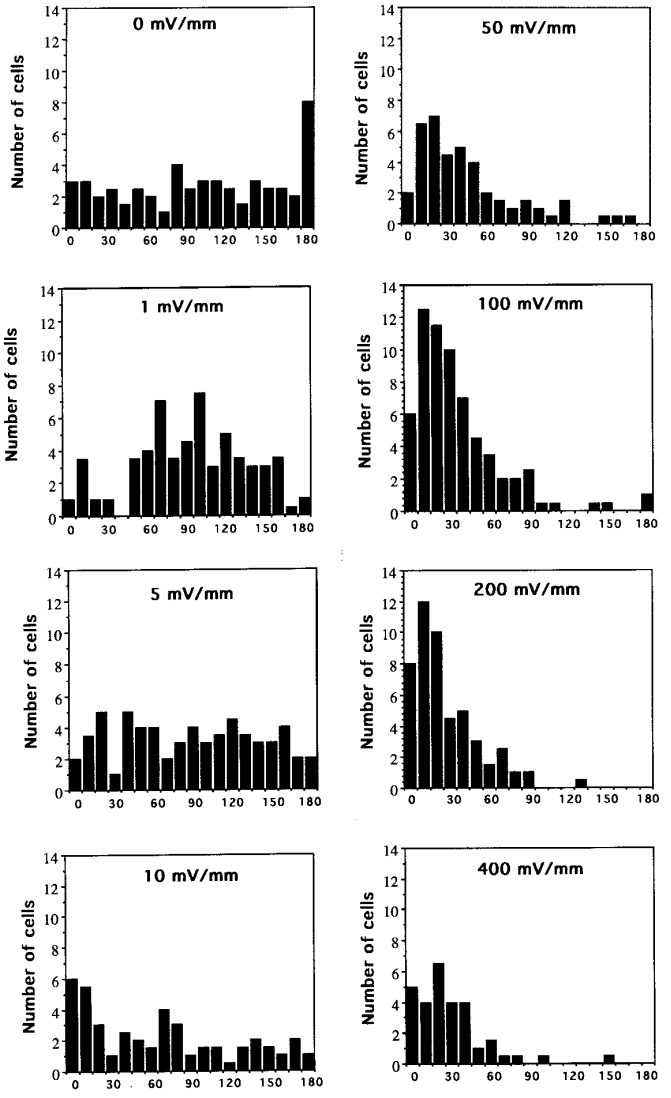

Fig. 3. Histograms showing the average number of cells whose position after migrating for 2.5 hours in one of eight different electric field strengths fell within given 10° sectors at an angle ϕ ±5° with respect to the direction of the imposed electric field (0°). Since this response is symmetrical about the 0-180° axis, the number of cells in each 10° sector between 5° and 175° was averaged with the number of cells in the symmetrical sector on the opposite side of the *y* axis and this average number was plotted. The cell number in the sectors centered on 0° and 180° represent the absolute number of cells which migrated within their respective 10° sectors (355°-5° and 175°-185°, respectively).

Cell groups

As the cell density increases in culture, keratinocytes exhibit an attraction for one another and migrate towards each other to first form groups of cells and then a sheet of cells. These groups of cells also exhibit galvanotaxis. In an electric field of 100 mV/mm, paired cells exhibit a galvanotactic response ($\langle\cos\phi\rangle = 0.74\pm0.05$) that is similar to that observed in single cells ($\langle\cos\phi\rangle = 0.80\pm0.03$) (Table 2). Those cell groups composed of greater than three cells showed a significant reduction in directional migration ($\langle\cos\phi\rangle = 0.57\pm0.09$). Track velocity and net cellular translocation were highest for single cells.

Locomotion

Stationary cells are induced to migrate when exposed to electric fields by forming cytoplasmic processes toward the cathode. Crescenteric cells follow the direction of the convex edge, while the trailing edge remains somewhat linear and free of cellular processes. Their movement is smooth and continuous. Circular cells often migrate with one tubular extension leading the cell forward. Polygonal cells follow the direction of lamellipodia; however, because these extensions are often facing three different directions, their movement is non-linear, often shifting from one direction to another. Elongated cells display three general types of movement depending on the electric field

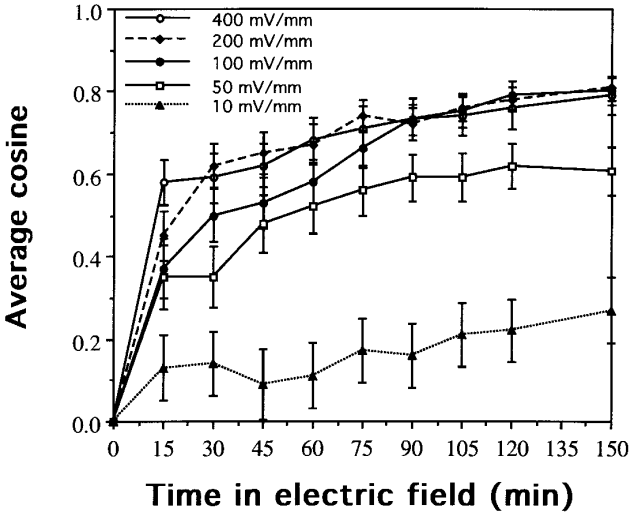

Fig. 4. The time course of the galvanotactic response. The average cosine of the translocation distribution was measured at 15 minute intervals for a two hour period and after 2.5 hours for the five field strengths indicated. The average cosine values represent cumulative directional translocation. The initial response time is shorter in the higher electric field strengths, with the greatest rate of change in migration direction occurring within the initial 15 minute period. The error bars indicate the s.e.m.

Table 1. Average direction of migration, net cellular translocation and migration velocity in imposed electric fields

Electric field strength (mV/mm)	Translocation distribution after 2.5 hours in field Mean cosine ϕ ± s.e.m. (n)	Net translocation (2.5 hours) Mean ± s.e.m. (μm)	Net translocation velocity* (2 hours) Mean ± s.e.m. (μm/min)	Track velocity† (2 hours) Mean ± s.e.m. (μm/min)	Migration index‡
0	–0.07±0.08 (93)	61±3	0.47±0.03	1.00±0.10	0.47
1	–0.06±0.06 (116)	48±3 (P<0.01)	0.36±0.02 (P<0.005)	0.80±0.02 (P<0.05)	0.45
5	0.03±0.06 (120)	63±3§	0.47±0.03§	1.00±0.02§	0.47
10	0.26±0.08 (77) (P<0.005)	58±3	0.40±0.03	0.60±0.03 (P=0.0005)§	0.66
50	0.61±0.06 (77) (P<0.0001)§	79±6 (P<0.01)§	0.60±0.04 (P<0.01)§	0.90±0.03§	0.66
100	0.80±0.03 (91) (P<0.0001)§	125±7 (P<0.0001)§	0.90±0.05 (P<0.0001)§	1.30±0.04¶	0.69
200	0.81±0.03 (90) (P<0.0001)	89±4 (P<0.0001)§	0.63±0.03 (P<0.001)§	0.80±0.03§	0.79
400	0.79±0.05 (51) (P<0.0001)	122±7 (P<0.0001)§	0.90±0.05 (P<0.0001)§	1.10±0.04§	0.82

*Net translocation velocity: net displacement from origin to endpoint at 2 hours/120 minutes.
†Track velocity: accumulated distance traveled between 15 minute points/120 minutes.
‡Migration index: net translocation velocity/track velocity calculated for a 2 hour period. A value of 1.0 indicates a linear pathway of migration from origin to endpoint; decreasing values indicate frequent changes in direction occurred during migration.
P values in parentheses = level of significance compared to the 0 mV/mm group.
§P<0.05 for the preceding group.
¶P<0.01 for all other values.

strength imposed on them: (1) in the lower electric field strengths (<100 mV/mm), the cells orient parallel to the direction of current, extend themselves toward the cathode, then retract the trailing end as the cell body rounds up. (2) Some elongated cells will pivot in place due to active lamellipodia at each end moving in opposing directions. (3) In electric field strengths above 100 mV/mm, elongated cells tend to align with their long axis perpendicular to the field and move towards the negative pole at a steep angle of 45-90° to their long axis, using each end to advance the cell toward the cathode.

Field-induced orientation of each cell's long axis

Human keratinocytes elongate and begin orienting their long axes perpendicular to the electric field beginning at field strengths of about 100 mV/mm. At this field strength, a majority of cells align approximately 45 degrees from the axis of the current. As the field reaches 200 and 400 mV/mm, the perpendicular alignment becomes more pronounced such that the long axis of a majority of cells lies approximately 80 degrees from the direction of current within 60 and 25 minutes, respectively. The onset of this response was observed within 15 minutes of field application for fields greater than or equal to 100 mV/mm with a more homogeneous and rapid response occurring as the field strength increased.

Proliferation

Cell division was observed in a few cells exposed to lower electric field strengths (≤50 mV/mm). Out of a total population of 715 cells observed in our study (including control groups), 483 were exposed to fields ≤50 mV/mm. Of this group of 483 cells, 4 single cells were observed to round up and divide during our experiments. These cells assumed a spherical shape while maintaining attachment to the substrate, underwent mitotic division, and then each daughter cell subsequently spread onto the collagen matrix and resumed independent migration. The entire process was completed within 30 minutes. Electric fields have been reported to enhance the rate of proliferation of fibroblasts (Gentzkow, 1993). Based on the limited number of cells that actually replicated during the course of our experiments, a mitogenic effect from DC electric field stimulation cannot be determined.

DISCUSSION

We have found that human keratinocytes are quite sensitive to imposed DC electric fields in the 10-400 mV/mm range, similar to those measured near wounds in guinea pig skin (Barker et al., 1982). This close correlation between the magnitude of endogenous electric fields and the optimal field strength for the galvanotaxis of keratinocytes in culture suggests that these cells might well use the endogenous electric fields near wounds as one of the earliest signals to guide migration into the wounded region.

Characteristics of the cellular response

Single cells vs grouped cells

Human keratinocytes exhibit galvanotaxis whether isolated, in

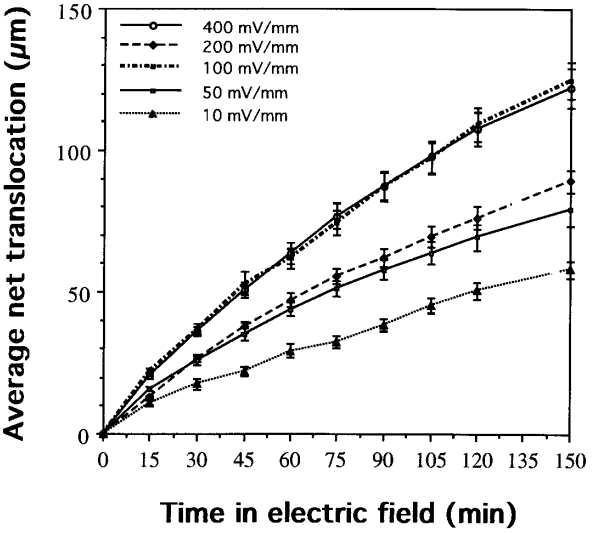

Fig. 5. The time course of the translocation response. The net translocation at 15 minute intervals for the initial 2 hour period and at 2.5 hours is shown for the five field strengths indicated. Net migration velocity is represented by the slope of each line and is highest for 100 and 400 mV/mm electric field strengths. The error bars indicate the s.e.m.

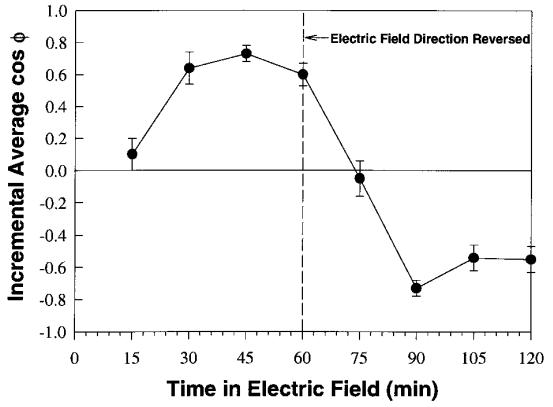

Fig. 6. The time course of the galvanotactic response in a 100 mV/mm electric field for one hour followed by a 180° field reversal for an additional hour. The incremental value of the average cosine of the translocation distribution at 15 minute intervals is shown for each field direction. The average cosine of cellular distribution within the first 15 minutes and the rate of approach to the final average cosine is similar in each period. The error bars indicate the s.e.m.

pairs, or in cell groups (≥3 cells). However, we observed that single keratinocytes are attracted to each other by some other mechanism (chemotaxis?) that is independent of the imposed field. Often, two cells that were close to each other would migrate towards each other and form a two-cell cluster before migrating towards the negative pole. A similar response was also observed in fish keratocytes by Cooper and Schliwa (1985) where they observed these cells to migrate towards the cathode as single cells, in cell clusters, or as cell sheets in DC fields ranging from 400-1,000 mV/mm. In electric fields greater than 1,000 mV/mm, single cells and clusters would separate from the confluent collection and migrate independently. The tendency for human keratinocytes to form groups was observed in all electric fields, particularly in the lower field strengths. The keratinocytes exposed to a field less than or equal to 1 mV/mm readily formed attachments with neighboring cells, becoming relatively immobile following union. In fields of 5-50 mV/mm, single cells often separated from their respective pairs or groups and resumed cathodal migration independently. With increasing field strength, cells tended to couple and group with migration continuing toward the cathode as a single unit. Analysis of the cellular translocation distribution shows that the directedness of migration in a field of 100 mV/mm is reduced as cells aggregate. The mean cosine of the translocation distribution for single cells was 0.80±0.03.

Table 2. Average cosine of the translocation distribution varies with the number of cell contacts

	Mean cosineϕ ± s.e.m. (n)	Track velocity (μm/minute ± s.e.m.)	Net translocation (μm)
Single cells	0.80±0.03 (91)	1.3±0.04†	124.8±6.5
Paired cells	0.74±0.05 (25)	1.1±0.06	119.9±9.5
Grouped cells	0.57±0.09 (30)	1.0±0.05	80.2±8.6†

*P<0.05 for single cells; †P<0.05 for all values; ‡P<0.005 for all values.

Paired cells migrated in a similar fashion as isolated cells (<cos ϕ> = 0.74±0.05), however, there was a decline in the average cos ϕ to 0.57±0.09 for cells in groups of 3 or more (Table 2). A contributing factor to this behavior may be that undetected forces triggering group formation influence the initial direction of migration until contact is achieved. Because all cells were isolated in the beginning of the experiments, the net pathway of migration includes the initial movement towards other keratinocytes during the group formation process. Once pairs or groups were formed, migration continued toward the negative pole, although in a less directed fashion.

Speed of cell movement

Imposed electric fields have a strong influence on directed migration but have little influence on the speed of migration. As reported by Woodley et al. (1993), and again demonstrated here in the control group, isolated human keratinocytes actively migrate on a matrix of collagen I in vitro without exposure to electric fields. The average migration velocity of 1.0±0.1 μm/minute (s.e.m., n=93) in the absence of a field corresponds to a displacement of approximately 1-1.5 cell diameters per hour. When exposed to a DC field of 100 mV/mm, the average migration speed reached a maximum of 1.3±0.04 μm/minute (n=100). No consistent relationship between migration speed and electric field strength is apparent. The fact that the directedness of migration (avg. cos ϕ) rises to a maximum without proportional and consistent changes in migration speed with increasing electric field strengths demonstrates that the cellular mechanisms determining the directedness and the speed of movement are independent of one another in cell motility (Gruler, 1993).

Response time

The response time (when half of the cells respond) for galvanotaxis in a field of 100 mV/mm is 14±1.1 (n=92) minutes, and the average cosine of the translational distribution exhibited significant directional movement within 15 minutes of field application (<cos ϕ> = 0.37±0.07). Keratinocytes exposed to fields greater than 100 mV/mm reacted more rapidly with cathodally-directed movement than those exposed to lower fields (based on the higher cosine values in the initial 15 minutes in 400 mV/mm field.). There is no apparent relationship between the cell density and response time to field application (data not shown). One interesting feature of this response time is that it differs from cell to cell. In avian neural crest cells it was found to be about 7 minutes long (Nuccitelli and Smart, 1989) and in granulocytes it is 8 seconds long (Franke and Gruler, 1990, 1994). In some cells the response time changes after exposure to electric fields. Stump and Robinson (1983) observed that amphibian neural crest cells responded with faster cathodal migration and a higher migration velocity after a reversal in the DC electric field and we found a similar behavior in avian neural crest cells (unpublished data) and avian fibroblasts (Erickson and Nuccitelli, 1984). This response time is probably indicative of some cellular process that is involved in signal detection and could be used in designing pulsed field application protocols that would differentiate between cell types attracted by the field.

Net cellular translocation and orientation

Analysis of net cellular translocation reveals that significantly

higher displacement occurs in response to fields of 100 mV/mm and 400 mV/mm compared to the lower fields or the control group. This occurs without proportional changes in overall migration velocity between the cell groups and suggests that in the lower electric fields, migration occurs in a less-directed, often tortuous pattern compared to a more linear migration pathway exhibited in electric fields of 100 mV/mm and above. Paired cells migrated a similar distance (120±10 μm) compared to isolated cells; grouped cells, however, traveled the least (80±9 μm).

In addition to the translocation response, we often see an orientation response with the cell's long axis oriented perpendicular to the electric field. Many investigators have reported that a perpendicular alignment of cells occurs with respect to the direction of the electric field upon application (Luther et al., 1983; Erickson and Nuccitelli, 1984; Cooper and Schliwa, 1985; Nuccitelli and Smart, 1989; Hinkle et al., 1981; Cooper and Keller, 1984). Luther et al. (1983) have shown that this is associated with a perpendicular alignment of stress fibers within the cell with a band of actin formation at the cathodal edge and distal tips of the amphibian epithelial cell. Since the magnitude of the voltage drop across the cell is proportional to the length of the cell parallel to the field lines, the cell will minimize the effect of the field by orienting its long axis perpendicular to the electric field lines. This perpendicular orientation is more prevalent in higher field strengths and suggests that cells actively orient their position in the field to minimize the magnitude of the voltage drop across the cell.

Comparison with previous galvanotaxis studies

The threshold of the galvanotactic response in human keratinocytes (0.5 mV/cell length) is comparable to physiologic thresholds observed in other cell types (reviewed by Nuccitelli, 1988). For example, avian neural crest cells initiate directional migration in DC electric fields as low as 7 mV/mm, or 0.4 mV across the cell length (Nuccitelli and Smart, 1989). Similar results were found in amphibian neural crest cells when a field of 10 mV/mm, or 0.7 mV per cell diameter, was imposed (Stump and Robinson, 1983). Cathodal migration can also be induced in embryonic quail fibroblasts when exposed to electric fields between 1 and 10 mV/mm, or 0.2 mV per cell. Additionally, in a study looking at electric field-induced directional growth, or galvanotropism, amphibian neurites responded to electric fields as low as 7 mV/mm by growing towards the negative pole of the imposed field (Hinkle et al., 1981).

Possible mechanisms of electric field detection by cells

The electrical properties of cells are determined largely by their plasma membrane which exhibits a very high electrical resistance to current flow. Ionic currents driven through the medium outside the cell by the imposed electric field will be forced to flow mainly around the cell due to this high transmembrane resistance. This increases the current density along the sides of the cell that are oriented parallel to the field lines, and enhances the local electric field in those regions by about 50% (Jaffe and Nuccitelli, 1977). This local field will exert an electrophoretic force on charged proteins and lipids in the plasma membrane which can act to redistribute such membrane components and indeed has been demonstrated to do so in many cell systems (Jaffe, 1977; Poo and Robinson, 1977; Orida and Poo, 1978; McLaughlin and Poo, 1981; Poo, 1981). Therefore, one popular mechanism for electric field detection involves the lateral electrophoresis of membrane proteins such as ion channels which could result in regional clusters of channels that could increase local fluxes of ions through the plasma membrane. For example, if Ca^{2+} channels were laterally electrophoresed toward the negative pole of the field, one would expect the rate of Ca^{2+} influx to increase in that region and this could signal the cell to form localized lamellipodia.

Another effect of imposed fields is the voltage across the membrane in the cellular regions facing the poles of the field. Since the imposed electric field shifts the relative voltage outside of the cell away from ground potential, the membrane potential will be increased on the side facing the positive pole and decreased on the side facing the negative pole. Therefore, in a 100 mV/mm field, the 6 mV drop across a 60 μm long cell would result in a hyperpolarization of 3 mV at the end of the cell facing the positive pole and a 3 mV depolarization at the end facing the negative pole. With a resting membrane potential of around −70 mV, this represents only a 4% change in local membrane potential, but could influence either the open probability of the channels in that region or the force driving ions through those channels.

Relevance to wound healing

The relevance of these results to wound healing is suggested by the presence of endogenous electrical potentials across intact human skin (Barker et al., 1982), and the existence of measurable electrical currents which exit wounded skin (Illingworth and Barker, 1980) and generate lateral electric fields which point towards the center of the wound in the region beneath the epidermis (Barker et al., 1982). Transcutaneous voltages in the range of 10-60 mV are maintained across intact epidermis largely by the Na^+/K^+-ATPase which pumps Na^+ across the epidermis. When a cut is made through the skin, current exits the low resistance pathway at the wound, resulting in the generation of lateral electric fields beneath the epidermis pointing towards the wound from all directions. This electric field beneath the epidermis has not been measured, but that above the epidermis has been measured in guinea pig by Barker et al. (1982). However, it is important to note that these measurements of lateral fields near wounds were made between the epidermis and the stratum corneum where the wound is positive with respect to lateral regions. The polarity of the lateral electric field vector beneath the epidermis is opposite to that above it and will direct suprabasal keratinocytes to migrate *towards the wound*, but the magnitude of the in vivo field in that region is unknown. These basal fields may be smaller than those measured between the living epidermis and the stratum corneum because the resistivity there is probably lower than it is beneath the stratum corneum. However, it is likely that the voltage gradient in this region will still be in the physiologic response range of 10-100 mV/mm.

Electric fields have also been measured in wounds of newt skin (Chiang et al., 1991), in bovine corneal wounds (Chiang et al., 1992), and in humans (Illingworth and Barker, 1980). Chiang et al. (1991) provided evidence for the significant role of intrinsic electric fields in the promotion of wound healing in the newt. In experiments that electrically reversed the polarity or abolished the intrinsic wound electric fields, the rate

of healing was reduced by about 15%, but increasing the local field did not speed up the healing process. This suggests that evolution has already optimized the cellular response mechanisms in healthy individuals but leaves open the possibility that certain disease states might result in reduced injury currents. In those cases increasing the local electric field near the wound might well improve the rate of wound healing. Indeed, there are several claims of enhanced wound healing by electric field application in the literature (Vanable, 1989; Gentzkow, 1993).

In summary, human keratinocytes migrating on collagen in tissue culture exhibit galvanotaxis when exposed to DC electric fields greater than 10 mV/mm. Directedness, net translocation, and migration velocity are maximum at 100 mV/mm. This optimal field strength found to elicit galvanotaxis is similar to that measured above the epidermis near wounds in guinea pig skin. Since the local electric field generated by the wound current appears immediately upon disruption of the epidermal layer, we propose that this lateral electric field beneath the epidermis generated by the skin battery provides the *earliest* signal to keratinocytes in that region to migrate towards the wound.

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