Entamoeba histolytica cell movement: A central role for self-generated chemokines and chemorepellents

Mehreen Zaki, Natalie Andrew, and Robert H. Insall*  

School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, United Kingdom

Entamoeba histolytica cells, the cause of amoebic dysentery, are highly motile, and this motility is an essential feature of the pathogenesis and morbidity of amoebiasis. However, the control of E. histolytica motility within the gut and during invasion is poorly understood. We have used an improved chemotaxis assay to identify the key extracellular signals mediating Entamoeba chemotaxis. The dominant responses we observe are caused by factors generated by E. histolytica cells themselves. Medium that has been conditioned by E. histolytica growth causes both chemokinesis and negative chemotaxis. The speed of random movement is more than doubled in conditioned compared with fresh medium, and cells move efficiently away from conditioned medium by negative chemotaxis. Ethanol, the product of Entamoeba glucose metabolism, is the principal component of the chemokinetic response. The closely related but nonpathogenic Entamoeba dispar shows no change in motility in response to conditioned medium implying that these responses are central to Entamoeba histolytica pathogenesis.

An estimated 50 million individuals suffer the severe morbidity associated with invasive Entamoeba histolytica infections, with an estimated 100,000 deaths annually (1). Parasite–host interactions that determine the course of infection, in particular asymptomatic colonization vs. symptomatic invasive disease, are largely still a mystery. It is, however, accepted that amoebic adherence to and contact-dependent killing of target cells, followed by phagocytosis, are key events (1, 2). The ability to interact with target cell surfaces is therefore a major process underpinning amoebic invasion of the human intestine. Consequently, research centered on E. histolytica cell surface receptors has great clinical promise. In particular, a role of chemotaxis seems likely. Zymosan activated C5a in human serum, hysed red blood cells, whole bacteria, components of the rat colon, N-acetylneuraminic acid (NANA) and NANA-containing compounds, fibronectin and fibronectin-derived fragments, and human TNF have all been shown to provide chemotactic stimuli (3–7).

E. histolytica motility and chemotaxis have been studied by using relatively few methods, including in haemocytometers (8), tube migration (9), and Boyden chamber assays (3). Under the first two conditions, cells have almost no resistance to their movement except substrate adhesion. During invasive disease, however, amoebae move in more restrictive conditions, similar to metastasis and extravasation. Under-agarose (under-agar) assays provide such an environment and have been used to study the motility and chemotaxis of a variety of cells, including neutrophils, macrophages (10, 11), and the free-living amoeba Dictyostelium (12), an evolutionary relative of Entamoeba (13). Under-agar assays have the added advantage of allowing moving cells to be visualized and parameters such as cell shape to be studied in detail.

To date, there appears to have been only one attempt at establishing an under-agar assay for studying E. histolytica motility (7). In this work, migratory distances were measured only after fixation and staining of cells. An improved under-agar assay for studying Dictyostelium chemotaxis under mechanically inhibited conditions has recently been reported (14). The assay allows high-resolution live imaging of cells, and movement is quantifiable for both individual and whole populations of cells.

We have, therefore, adapted this assay for studying Entamoeba chemotaxis. We have in the course of this study discovered that components of E. histolytica-conditioned culture medium are key determinants of E. histolytica cell motility. The response to conditioned medium (CM) includes an increase in both speed and negative chemotaxis. Crucially, the commensal (nonpathogenic) Entamoeba dispar does not show any such behavior, implying that negative chemotaxis may play an important role in E. histolytica pathogenesis. These findings, therefore, have clinical implications, in particular suggesting a mechanism that drives E. histolytica cell migration away from the intestinal lumen and mucous layer and toward the underlying epithelium.

Results

E. histolytica Chemotaxis Is Dominated by Cell-Derived Stimuli. We optimized the three-trench under-agar assay used by Dictyostelium researchers (14) for Entamoeba (see Supporting Text, which is published as supporting information on the PNAS web site, for details). LYI-S-2 medium and neat serum, which were found to attract Entamoeba in earlier studies (3), were included as internal controls. As expected, the migration distance was greatest toward LYI-S-2 (Table 1 and data not shown), which was 1.5 times greater than that seen with the MM-1 control (Table 1 and Fig. 1Bi). The response to serum was unexpectedly negligibly different from the control (Table 1 and Fig. 1Ai). LPA and cAMP, which are strong chemoattractants for Dictyostelium, were not chemoattractants for Entamoeba under any conditions (Table 1 and data not shown).

Dictyostelium cells in under-agar assays never move out of the well unless a chemoattractant is present (ref. 14; corroborated by our observations; data not shown), but we consistently found that

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<tr>
<th>Chemotactic stimulus</th>
<th>Migration distances, μm</th>
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<tr>
<td></td>
<td>I margin</td>
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<tr>
<td>LYI-S-2</td>
<td>244.5 ± 50.8</td>
</tr>
<tr>
<td>Serum</td>
<td>142.8 ± 12.3</td>
</tr>
<tr>
<td>MM-1</td>
<td>150.8 ± 16</td>
</tr>
<tr>
<td>LPA</td>
<td>163.7 ± 11.7</td>
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<tr>
<td>cAMP</td>
<td>152.5 ± 3.2</td>
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Numbers represent mean values of two experiments at 2 h.

*To whom correspondence should be addressed. E-mail: r.h.insall@bham.ac.uk.

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E. histolytica cells exited from their respective troughs in the absence of a chemotactic gradient. The mean migratory distances at the outer margins of the trough (which, in the original version of this assay, serve as a negative control, with no cells normally migrating; see Figs. 9 and 10, which are published as supporting information on the PNAS web site; see ref. 14) were comparable to those observed in response to serum and MM-1 at the inner margins (Table 1; Fig. 1 Ai and Bi). Assays in which the agarose was prepared by using LYI-S-2 or medium containing 2% serum resulted in similar migratory patterns (data not shown). This result implies that the under-agar migration of E. histolytica at the outer margins and in controls was in response to stimuli present in the trough.

The most likely source for the unknown stimuli was expected to be either depletion of components of the medium in which the cells were suspended or release of E. histolytica-derived component(s). Diffusion of these components could establish a gradient directing cell migration away from the origin of the stimulus, in this instance, the trough. In addition, these cell-derived components could also stimulate random cell movement (chemokinesis).

**Components of CM Increase Cell Speed.** To test the hypothesis that E. histolytica migration is driven by amoeba-derived components, we compared the effects on cell motility, speed, and direction of fresh LYI-S-2 medium and LYI-S-2 medium conditioned by 96 h of E. histolytica growth. Cell speed was determined by using a Dvorak–Stotler chamber, which allows detailed observations of cells while controlling medium exchange (Fig. 10). E. histolytica cells were perfused with fresh or CM and allowed to adapt to the change in environment for 20–30 min, then time-lapse images were recorded and cell speed was determined. The results clearly show that components of CM increase cell speed.

The average speed in centrifuged CM (23.7 ± 2.3 μm/min) was about twice that seen in fresh medium (FM; 11.6 ± 1 μm/min; P < 0.001; Fig. 2A). With few exceptions, cells in FM also showed decreased net translocation from the point of origin (Movies 1–7, which are published as supporting information on the PNAS web site). In contrast, the majority of the cells in CM moved a substantial distance (Movie 2). The relatively gentle centrifugation might not have been sufficient to remove all particulate matter, so cell speed was also determined by using 0.2 μM filtered CM (Fig. 2B). The speed in filtered CM remained significantly (P < 0.001) greater than in FM, showing that the active components are soluble. The

**Fig. 1.** Under-agar migration of E. histolytica. Amoebae suspended in control medium MM-1 are shown migrating from the inner “I” margin of the peripheral trough toward (Ai) serum (test) or (Bi) MM-1 (control) in the central well. Amoebae also migrated out of the peripheral troughs from the outer “O” margins in both the test (Ai) and control (Bi) plates. Images taken at 2 h and representing one of two experiments are shown. Arrows mark the trough margins (continuous (I) and broken (O)), as well as showing the direction of cell migration. (Scale bar, 50 μm.)

**Fig. 2.** Determination of E. histolytica cell speed using the Dvorak–Stotler chamber. Cell speed was determined for individual amoebae in FM or CM. Three to four experiments were done for each medium type. Values represent the mean of 20 cells. Error bars are standard error. FM vs. centrifuged CM (cCM, A), filtered CM (fCM, B), frozen CM (frCM, C) and CM and dialyzed CM (dCM, D). *, P < 0.01; ***, P < 0.001.

**Fig. 3.** Determination of the direction of E. histolytica migration using the Dunn chemotaxis chamber. Direction of the response (CI) of individual amoebae to and from FM or CM was determined. All experiments were performed in duplicate. Cell tracks for a number of amoebae (n) from both experiments were plotted on the same vector diagram. Vector diagrams are all oriented so that (0, 0) marks the starting point of the track, and the position of the outer well of the Dunn chamber is to the right (black arrows). Response of cells seeded in FM was tested to (A) FM (n = 12; CI = +0.05) and (D) CM (n = 10; CI = −0.12). Response of cells seeded in CM was tested to CM (n = 10; CI = −0.05) and (B) FM (n = 14; CI = +0.34, C).
average speed in LYI-S-2, which had been incubated at 37°C for 96 h without cells, was comparable to that seen with FM (data not shown).

**CM Factors Are Stable and Dialyzable.** To further characterize the nature of the active components of CM, aliquots of CM were frozen at −20°C for 1 week and 1 and 5 months. Aliquots were thawed at designated time points, and cell speed was determined in comparison with FM. Fig. 2C represents the increase in average cell speed with CM that had been frozen for 5 months (P < 0.001) vs. FM. No significant difference was observed between the aliquots frozen for 1 week and 1 and 5 months (15.7 ± 0.9, 17.8 ± 1.4, and 16.7 ± 0.84 μm/min, respectively). However, a significant (P < 0.01) decrease was seen with frozen CM when compared with earlier results (centrifuged and filtered). This loss in activity does not appear to be affected by the duration of the freezing but rather by the process of freezing and/or thawing itself.

Finally, cell speed was also compared between FM, CM, and dialyzed CM (Fig. 2D). To prepare the dialyzed fraction, CM was dialyzed against two changes of 10X volumes of FM at 4°C over a 24-h period by using 12- to 14-kDa Visking dialysis tubing (Medicell International, London, U.K.). The average speed of *E. histolytica* in dialyzed CM was comparable to that seen with FM, and undialyzed CM speed was significantly (P < 0.01) greater than both FM and dialyzed CM. The active components are thus smaller than 12–14 kDa in size.

**CM Causes Chemotaxis as Well as Chemokinesis.** We have clearly shown that CM components cause chemokinesis. However, our results from the under-agar assay suggested a chemotactic response. We therefore studied amoebic responses to CM by using Dunn chambers, which allow cell direction to be analyzed under defined conditions (15, 16). As expected, cells suspended in homogenous medium moved randomly, and cells moved more slowly in FM than in CM (Fig. 3A and B and Movies 3 and 4). In gradients of FM and CM, cells moved directionally, showing that CM induces chemotaxis as well as chemokinesis.

When cells were seeded in CM, they nearly uniformly migrated toward FM [chemotactic index (CI) = +0.34] in the outer well of the Dunn chamber (Fig. 3C and Movie 5). When the experimental setup was reversed, the amoebae seeded in FM were clearly repelled (CI = −0.12) by the CM in the outer well of the Dunn chamber (Fig. 5).

### Table 2. Under-agar migratory responses of *E. histolytica* to and from different media using the modified single trough assay

<table>
<thead>
<tr>
<th>Chemotactic stimulus</th>
<th>Migration distances, μm</th>
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<tbody>
<tr>
<td>MM-1Gluc → FM</td>
<td>506.4 ± 61.2</td>
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<tr>
<td>FM* → CM</td>
<td>80.13 ± 8.4</td>
</tr>
<tr>
<td>FM* → FM</td>
<td>211.5 ± 23.5</td>
</tr>
<tr>
<td>CM* → CM</td>
<td>129.7 ± 12.3</td>
</tr>
<tr>
<td>MM-1Gluc1→ CM</td>
<td>14 ± 7.3</td>
</tr>
<tr>
<td>MM-1Gluc1→ CM</td>
<td>665.9 ± 26.8</td>
</tr>
<tr>
<td>CM* → MM-1Gluc1</td>
<td>721.7 ± 21.5</td>
</tr>
<tr>
<td>MM-1Gluc1→ FM</td>
<td>266.6 ± 26.3</td>
</tr>
<tr>
<td>FM* → MM-1Gluc1</td>
<td>205.3 ± 47</td>
</tr>
</tbody>
</table>

Numbers represent mean values of three experiments at 6 h. FM (fresh LYI-S-2 medium); CM (96-hr-old *E. histolytica*-conditioned LYI-S-2 medium); MM-1Gluc1 (MM-1 medium without glucose). Medium in the trough is designated by (*), whereas the arrow points towards the medium in the agarose layer. Statistical comparisons (ω) show a significant increase in distance migrated; P < 0.01 (also see Fig. 5).

### Fig. 4. Determination of the direction of *E. histolytica* migration using the under-agar chemotaxis assay. Direction of the response of a population of amoebae to and from FM or CM was determined. Images taken at 6 h and representing one of three experiments are shown. Response of amoebae in CM was tested to FM (A) and CM (D). Response of amoebae in CM was tested to CM (B) and FM (C). The broken arrow marks the trough margins as well as showing the direction of cell migration. C depicts the maximum width of the visual field at this magnification and not the furthest migrated (leading) cell(s). (Scale bar, 50 μm.)

### Fig. 5. Determination of *E. histolytica* migration (under-agar) using MM-1Gluc− as the control. Direction of the response of a population of amoebae to FM or CM was determined. Images taken at 6 h and representing one of three experiments are shown. Response of amoeba in MM-1Gluc− was tested to MM-1Gluc− (A), CM (D), and FM (E). Response of amoebae in CM was tested to MM-1Gluc− (B) and finally, the response of amoebae in FM was tested to MM-1Gluc− (C). The broken arrow marks the trough margins as well as showing the direction of cell migration. B depicts the maximum width of the visual field at this magnification and not the furthest migrated (leading) cell(s). Statistical comparisons between B and A and E and A show a significant increase in distance migrated; P < 0.01. (Scale bar, 50 μm.)
chamber (Fig. 3D and Movie 6). These results could indicate either positive chemotaxis toward FM or negative chemotaxis away from the CM in which they were seeded.

Positive vs. Negative Chemotaxis. To distinguish whether amoebae were in fact attracted to FM or repelled by CM, we adopted a simplified variation of the under-agar assay used earlier. This single-trough assay (Fig. 9B) measures chemotactic gradients formed between the medium in the trough and the medium in the agarose. We first tested this assay to see whether it would support the Dunn chamber analysis (Fig. 3). As before, cells in CM in the trough migrated significant distances under agarose containing FM, whereas cells in FM barely moved under agarose containing CM (Table 2 and Fig. 4 C and D), when the experimental setup was reversed. Amoebae were also able to migrate out of their respective troughs to different degrees when in homogenous FM and CM (Table 2 and Fig. 4A and B). The under-agar assay therefore mimics the results achieved with the Dunn chamber.

We then tested both FM and CM against the MM-1 control (17) in all possible combinations by using the single-trough under-agar assay. The distance migrated was similar for all of the test settings, in all possible combinations by using the single-trough under-agar assay. This and our earlier observation (Fig. 1 Ai and Bi) suggested that MM-1 was sufficient to support E. histolytica-CM chemotaxis. However, a modification of MM-1 without glucose (the only ingredient unnecessary for short-term viability, attachment and motility; ref. 17) provided a sharp contrast to all of the other tested media. MM-1Gluc was also able to support short-term viability, attachment and motility. Our earlier observations (Fig. 3 and 4) and those shown in previous work (7) were therefore presumably caused by a combination of repulsion from component(s) of CM and by attraction to component(s) of MM-1Gluc. MM-1Gluc is thus an ideal basis to distinguish positive and negative chemotaxis.

We tested the migration of amoebae in different combinations of MM-1Gluc−, CM and FM in the well, and the agarose. The results were complex, but the clearest result was obtained with cells in CM and MM-1Gluc− agarose. Cells migrated rapidly away from the trough into the agarose (P < 0.01; Table 2 and Fig. 5B). Because MM-1Gluc− itself is not a chemoattractant, this clearly demonstrates that cells in glucose-containing medium make a negative chemoattractant for E. histolytica.

Amoebae in MM-1Gluc− migrated toward either CM or FM (Table 2 and Fig. 5D and E), somewhat more toward the FM (P < 0.01). These effects were consistently less pronounced than when cells move away from CM. We believe that the migration of amoebae toward CM reflects a somewhat diluted response to the attractive components of LYI-S-2 culture medium, which are presumably partially but not yet completely depleted from the CM.

Our earlier observations (Fig. 3 and 4) and those shown in previous work (7) were therefore presumably caused by a combination of repulsion from component(s) of CM and by attraction toward FM. Fig. 5 shows that negative chemotaxis from CM has a greater effect than the positive chemotaxis toward medium seen previously and is thus a principal determinant of cell motility under normal experimental conditions.

Ethanol Is a Key Component of CM. Comparison of E. histolytica responses to LYI-S-2, MM-1, and MM-1Gluc− strongly suggested that the active components of CM were being generated as a consequence of glucose metabolism. E. histolytica lives by anaerobic...
in vitro identical chemokinesis and chemotaxis.

between the three media types tested (Fig. 8 in fresh 96-h-old
in their genetic background, cell biology and host range; humans are
determinant of the CM responses.

by physiological ethanol levels, showing that ethanol is a principal
the chemotactic effect of CM can therefore be completely recreated
CM contained 128
our estimate of ethanol concentrations was correct, we assayed
suggest that the directional response is random. To confirm that
ethanol is equal to that seen with CM in both Dvorak–Stotler and
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sequence comparisons have demonstrated 95% identity in coding

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are similar

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motility. It therefore seems that the extracellular signals controlling

E. histolytica motility. This changes in E.

E. histolytica pathogenesis.

Discussion

Parasite motility plays an important role in invasive amoebiasis
E. histolytica motility has been analyzed by using a limited
in viva environment that the amoebae are likely to be confronted
with during invasive disease. For invasive disease to occur the
protective mucosal barrier must be broken allowing contact between E. histolytica and the underlying epithelial cells. Mech-

E. histolytica and E. dispar motility are distinct, and the central role
of cell-derived repellents is specific to the pathological species.

Needless to say this has profound implications for the basis of E.

Responses to CM Correlate with Pathogenicity. These changes in E.
histolytica motility in response to accumulation of amoebic metabol-
ites have important clinical implications. In viva chemokinesis and
chemotaxis could be triggers that induce amoebic migration away
from the host’s intestinal lumen and protective mucous barrier
toward the underlying epithelium. To test this hypothesis, we
decided to see whether the commensal (nonpathogenic) E. dispar,
which is E. histolytica’s closest known relative (19), showed similar
chemokinesis and chemotaxis. E. histolytica and E. dispar are similar
in their genetic background, cell biology and host range; humans are
the only host for both, and therefore a comparison between the two
provides an important means for identifying E. histolytica-specific
virulence and pathogenicity mechanisms.

The Dvorak–Stotler chamber was used to determine cell speed in
fresh 96-h-old E. dispar-CM and simulated-CM containing 100
mM ethanol. We found no significant difference in cell speed
between the three media types tested (Fig. 8A and Movie 7). This
was a striking result. Both Entamoeba species are maintained under
identical in vitro culture conditions in our laboratory (see experi-
ental Procedures), and there is no reason to believe that the two
amoebae do not share the same metabolic pathway. There is no
evidence of significant differences in gene content; differences
described in the literature are uncommon and specific. Limited
sequence comparisons have demonstrated 95% identity in coding
and 80% in noncoding regions and, where studied, even the order
of the genes on the chromosomes of the two organisms has been
found to be identical (20).

These data imply that E. dispar is not responsive to the active
components of E. dispar-CM rather than that there are differences
in the metabolic pathways of E. dispar and E. histolytica. To confirm
this, E. dispar cell speed was also tested in the presence of E.
histolytica CM (Fig. 8A). Again, there was no significant difference
between the motility of cells in FM and CM.

Having observed that the CM components inducing chemoki-
nesis and chemotaxis are different, we decided to look at E. dispar
chemotaxis by using the modified single-trough under-agar assay.
Under-agar migration of E. dispar to and from FM, E. dispar-CM,
and CM was studied in all possible permutations. E. dispar cells
did not migrate out of the trough in any of the tested combinations (Fig.
8B and data not shown). As described earlier and in ref. 14, this is
the expected result if no chemotactic stimuli are present. Thus, CM
is neither a chemokine nor a chemoattractant for E. dispar, in
striking contrast to the dominant role it plays in E. histolytica
motility. It therefore seems that the extracellular signals controlling
E. histolytica and E. dispar motility are distinct, and the central role
of cell-derived repellents is specific to the pathological species.

Needless to say this has profound implications for the basis of E.
histolytica pathogenesis.

carbohydrate metabolism (18), generating ethanol from glucose by
glycolysis. We therefore compared the effects of ethanol on cell
speed and direction by mixing varying concentrations of ethanol
with FM. Cell speed was determined by using the Dvorak–Stotler
chamber, whereas the direction of E. histolytica response to and
from the different test media was studied with the Dunn chamber.

We found that medium containing ethanol at physiological
concentrations (100 mM) causes faster movement (P < 0.01; Fig.
6). The average speed of cells in medium containing 100 mM
ethanol is equal to that seen with CM in both Dvorak–Stotler and
Dunn chamber assays. CI values of close to zero in both Fig. 7A and
B suggest that the directional response is random. To confirm that
our estimate of ethanol concentrations was correct, we assayed
ethanol levels in CM by using a colorimetric enzymatic assay. A 96-h
CM contained 128 ± 5.5 mM ethanol. The chemokinetic but not
the chemotactic effect of CM can therefore be completely recreated
by physiological ethanol levels, showing that ethanol is a principal
determinant of the CM responses.

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“accidentally.” In any case, comparison of the two species could help elucidate the specific mechanisms involved in the pathogenicity of *E. histolytica*.

It has so far been difficult to correlate the different pathogenic potentials of the two amoebas with the presence or absence of any specific virulence factors. Although higher activities of some proteins and a few *E. histolytica*-specific molecules may account for some of *E. histolytica*’s invasive capacity, by and large the molecules considered important for host tissue destruction are present in both and seem to be used primarily for colonizing the human gut and degrading nutrients (27). In this work, we suggest a previously undescribed difference. This work provides an account of a physiologically significant difference between *E. histolytica* and *E. dispar* motility.

That *E. dispar* does not mimic any of the changes in motile behavior exhibited by the human pathogen *E. histolytica* has important clinical implications. This behavior could be an *E. histolytica*-specific trigger that promotes migration of amoebae away from the host’s intestinal lumen and protective mucous barrier, which they had hitherto been colonizing harmlessly, and toward the underlying epithelium, marking the onset of invasive disease. Thus the difference between pathogenic and nonpathogenic amoebas could be rooted in alternative behavior, rather than in particular proteins or molecules that harm the host.

**Experimental Procedures**

**Cell Cultures.** Axenic *E. histolytica* strain HM-1:IMSS clone 9 and *E. dispar* strain SAW760 were maintained in LYI-S-2 medium (28), supplemented with 15% heat-inactivated adult bovine serum (Sigma–Aldrich, St. Louis, MO) at 36°C. For experimental use, trophozoites from mid-log phase (48–72 h) were harvested by chilling the culture tubes for 5 min in an ice-water bath followed by centrifugation at 275 × g for 5 min. Ethanol levels were assayed in test media using the Ethanol Assay Kit (Biomedical Research Service Center, State University of New York, Buffalo, NY; Cat. No. A-111).

**Under-Agar Chemotaxis Assay.** The under-agar assay used to study the motile behavior of Dicyostelium (14) was adapted for *Entamoeba*. In a typical assay, 14 ml of 0.75% Seakem GTG agarose dissolved in MM-1 (17) was poured into 100-mm plastic Petri dishes and allowed to solidify for 1 h at room temperature. Three 2-mm-wide troughs were cut 5 mm apart (4 cm length), as shown in Fig. 9A. Chemoattractant (200 μl) or MM-1 was added to the center trough and allowed to form a gradient for 1 h at room temperature. Approximately 1 h before the assay, the culture medium from amoebae to be used in the experiment was removed and replaced with MM-1 at 36°C. Amoebae were harvested, numbers were adjusted to 2.5 × 10^7 amoebae/ml in MM-1, and 200 μl of this cell suspension was added to the peripheral troughs. Plates were maintained at 36–37°C in a humidified atmosphere containing 5% CO_2 for the duration of the assay.

In some cases, a modified version of the under-agar assay was used. Chemoattractant to be tested was mixed with the cooled agarose mixture before being poured into the Petri plates, and a single trough was cut out for the amoebic suspension (Fig. 9B). Thus, the chemoattractant to be tested is present uniformly throughout the agarose. The diffusion of medium between the agarose and the trough establishes the gradient and drives chemotaxis. The results for assays shown here were comparable between the original and modified under-agar versions.

**Dvorak—Stotler-Controlled Environment Culture Chamber Assay.** Cell motility was also studied by using a Dvorak–Stotler-controlled environment cell culture chamber (Fig. 10), positioned on a microscope stage fitted with a heating chamber maintained at 36°C and perfused with prewarmed LYI-S-2 using a Model YA-12 syringe pump (Yale Apparatus, Wantagh, NY). Culture medium from amoebae to be used in the experiment was changed with fresh LYI-S-2 before being perfused into the chamber and allowed to adhere for 30 min, then 5 ml of LYI-S-2 was passed over the cells, allowing complete medium exchange within the chamber and washing away any detached cells. Test medium was perfused through, the pump was switched off, and cells were allowed to rest and adapt for 20–30 min. Cell motility was observed, recorded, and analyzed (see below).

**Analysis of Cell Motility.** All cell images were recorded at 36°C on a Model ULWCD 0.30 microscope (Olympus, Tokyo, Japan), fitted with 10× phase-contrast optics and heated chambers. Images were recorded from both inner (I) and outer (O) margins of both the peripheral wells (Fig. 9). Migratory responses of whole-cell populations to a chemoattractant stimulus were calculated as follows. First, the average distance traveled by the three leading cells from the trough edge was determined at each time point; means were then calculated for a minimum of two independent experiments. Time-lapse images were generated with frames captured every 12 sec for a period of 15 min. Only cells that remained in the field of view and that did not interact with another cell throughout the length of the movie were considered for the final analysis. Individual cell speeds were determined and tracks generated from the time-lapse images using ImageJ. The CI was calculated as the ratio between the distance traveled directly toward the medium in the outer well of the Dvorak chamber and the total distance traveled over the duration of the time-lapse movies (Movies 1–7). All deviations from the mean are reported as standard error and are checked for significance by using Student’s *t* test and a one-way ANOVA.

We thank Dr. Graham Clark for help in establishing *E. histolytica* and *E. dispar* cultures in our laboratory and for constructive comments on the manuscript. This work was supported by Grant INCO-DEV (ICA4CT-2001-10073) in the Fifth Framework Program of the European Union. We thank Dr. Nancy Guillen for initiating and organizing this grant, for introducing the Insall lab to *Entamoeba*, and for continued advice throughout the project. This work was also supported by the Biotechnology and Biological Sciences Research Council (BBE/RSC; Project Grant 6/G17939). R.H.I. is supported by a Medical Research Council Senior Research Fellowship.
- Amoebae
- Medium

- Syringe Pump
- Spacer - Outlet
- Spacer - Inlet

- Snap Ring
- Compression Ring
- Pressure Plate
- Teflon Seal
- 2nd Coverslip
- Teflon Seal
- 1st Coverslip*
- Teflon Seal
- Base/ Holder