

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

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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 *E. histolytica* pathogenesis.

invasion | negative chemotaxis | pathogenesis

An estimated 50 million individuals suffer the severe morbidity associated with invasive *Entamoeba histolytica* infections, with $\approx 100,000$ 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, lysed 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

Table 1. Under-agar migratory responses of *E. histolytica* to some chemoattractants using the original three-well assay template

Chemotactic stimulus	Migration distances, μm	
	I margin	O margin
LYI-S-2	244.5 $\pm$ 50.8	154.0 $\pm$ 19.9
Serum	142.8 $\pm$ 12.3	146.4 $\pm$ 9.8
MM-1	150.8 $\pm$ 16	169.0 $\pm$ 7.4
LPA	163.7 $\pm$ 11.7	159.6 $\pm$ 7.6
cAMP	152.5 $\pm$ 3.2	141.2 $\pm$ 5.6

Numbers represent mean values of two experiments at 2 h.

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-trough 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. 1*Bi*). The response to serum was unexpectedly negligibly different from the control (Table 1 and Fig. 1*Ai*). 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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Abbreviations: CI, chemotactic index; FM, fresh medium; CM, conditioned medium; under-agar, under-agarose.

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

Chemotactic stimulus	Migration distances, μm
CM* $\rightarrow$ FM	506.4 $\pm$ 61.2
FM* $\rightarrow$ CM	80.13 $\pm$ 8.4
FM* $\rightarrow$ FM	211.5 $\pm$ 23.5
CM* $\rightarrow$ CM	129.7 $\pm$ 12.3
MM-1Gluc ⁻¹ * $\rightarrow$ MM-1Gluc ⁻¹	14 $\pm$ 7.3
MM-1Gluc ⁻¹ * $\rightarrow$ FM	665.9 $\pm$ 26.8 ω
CM* $\rightarrow$ MM-1Gluc ⁻¹	721.7 $\pm$ 21.5 ω
MM-1Gluc ⁻¹ * $\rightarrow$ CM	266.6 $\pm$ 26.3
FM* $\rightarrow$ MM-1Gluc ⁻¹	205.3 $\pm$ 47

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-1Gluc⁻¹ (MM-1 medium without glucose). Medium in the trough is denoted 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).

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 $\mu\text{m}/\text{min}$, 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 $10\times$ 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

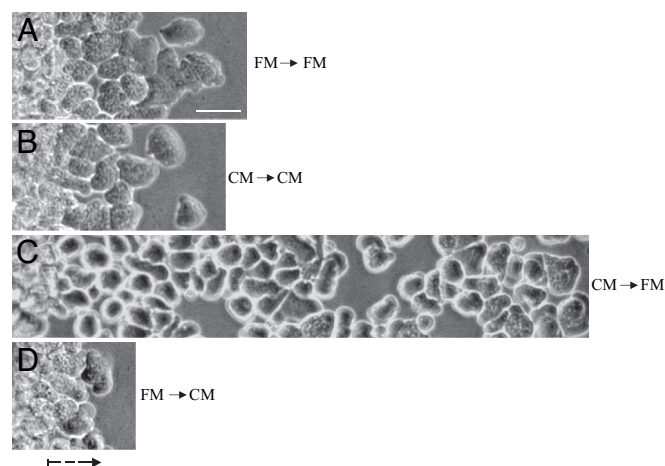


Fig. 4. Determination of the direction of *E. histolytica* migration using the under-agar chemotaxis assay. Direction of the response of a population of amoebae to and from FM or CM was determined. Images taken at 6 h and representing one of three experiments are shown. Response of amoebae in FM was 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 .)

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

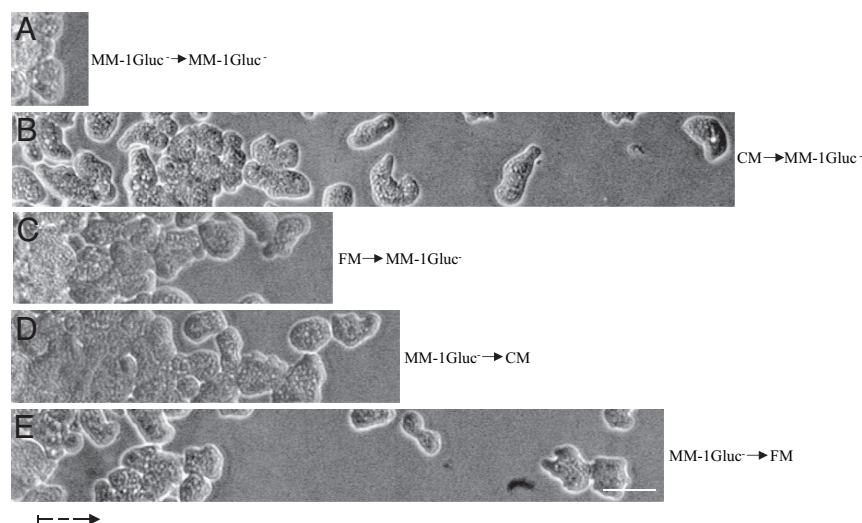


Fig. 5. Determination of *E. histolytica* migration (under-agar) using MM-1Gluc⁻ as the control. Direction of the response of a population of amoebae to FM or CM was determined. Images taken at 6 h and representing one of three experiments are shown. Response of amoebae in MM-1Gluc⁻ was tested to MM-1Gluc⁻ (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. 4C 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, including the control (data not shown). This and our earlier observation (Fig. 1*Aii* and *Bii*) 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⁻ caused little if any under-agar migration of control cells (Table 2 and Fig. 5A). This shows that MM-1Gluc⁻ neither contains chemoattractants that can be depleted nor supports the generation of cell-derived chemorepellents. 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

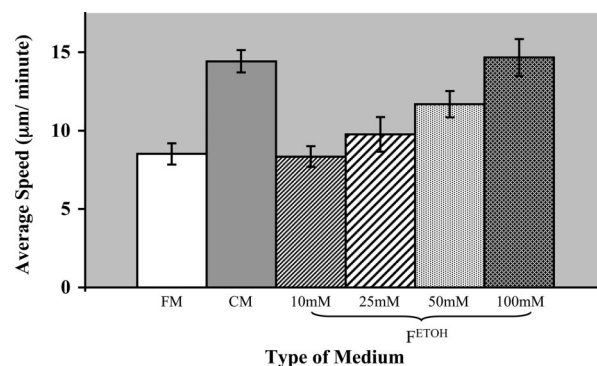


Fig. 6. Determination of *E. histolytica* cell speed in simulated-CM using the Dvorak–Stotler chamber. Cell speed was determined for individual amoebae in FM, CM, and simulated-CM (F^{ETOH}) containing varying (10, 25, 50, and 100 mM) amounts of ethanol. Three to four experiments were done for each medium type. Values represent the mean of 10 cells. Error bars are standard error. Statistical comparisons between 100 mM F^{ETOH} and FM and CM and FM show a significant increase in cell speed; $P < 0.01$.

(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

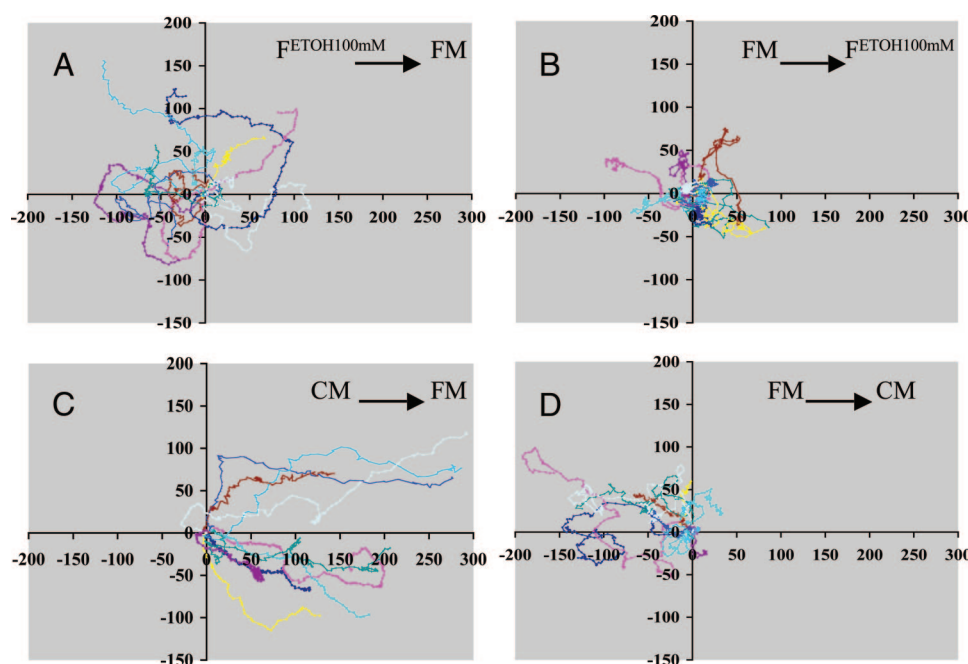


Fig. 7. Determination of the direction of *E. histolytica* migration in simulated CM using the Dunn chemotaxis chamber. Direction of the response (CI) of individual amoebae to and from FM, CM, and simulated CM containing 100 mM ethanol (F^{ETOH}100mM) was determined. All experiments were performed in triplicate. Cell tracks for 10 cells from all three 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). Responses of cells seeded in F^{ETOH}100mM (CI = <0.05, A) or CM (CI = +0.4, C) were tested to FM. Response of cells seeded in FM was tested to F^{ETOH}100mM (CI = <0.05, B) and CM (CI = -0.14, D).

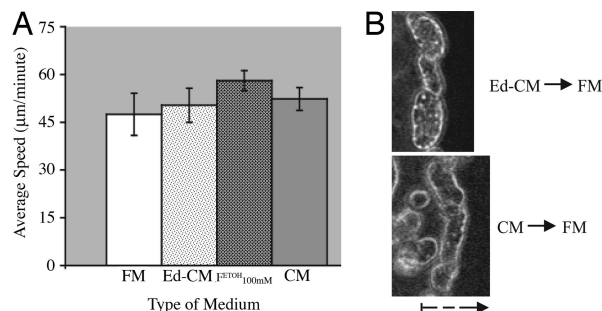


Fig. 8. Determination of *E. dispar* cell speed and direction of migration. (A) The Dvorak–Stotler chamber was used to determine cell speed of individual amoebae in FM, *E. dispar*-CM (*Ed*-CM), simulated CM containing 100 mM ethanol ($E_{\text{ETOH}100}$ mM), and CM. Three to four experiments were done for each medium type. Values represent the mean of 10 cells. Error bars are standard error. Statistical comparisons revealed no significance. (B) Under-agar response of amoebae in (i) *Ed*-CM and (ii) CM was tested to FM. Images taken at 6 h and representing one of three experiments are shown. The broken arrow marks the trough margins as well as showing the direction of cell migration. (Scale bar, 50 μ m.)

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.

Responses to CM Correlate with Pathogenicity. These changes in *E. histolytica* motility in response to accumulation of amoebic metabolites have important clinical implications. *In vivo* 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. S4 and Movie 7). This was a striking result. Both *Entamoeba* species are maintained under identical *in vitro* culture conditions in our laboratory (see *Experimental 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 chemokinesis 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.

Discussion

Parasite motility plays an important role in invasive amoebiasis (21). *E. histolytica* motility has been analyzed by using a limited number of methods (3, 7–9), few of which mimic the restrictive *in vivo* 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. Mechanisms that allow this are not fully understood. It appears, however, that both physical forces and chemical signals are involved (22–24). To this end, we have adapted an under-agar chemotaxis assay (14) to study *Entamoeba* motility and migration under mechanically inhibited conditions. The assay has been shown to be reproducible, allows live imaging of cells in two dimensions and the movement is quantifiable.

We have, in the course of this analysis, shown that components of *E. histolytica*-conditioned culture medium modulate *E. histolytica* motility. *E. histolytica* displayed both negative chemotaxis and chemokinesis when exposed to the CM. Ethanol, a principal end product of anaerobic carbohydrate metabolism (18) is a key determinant of the observed responses. This is not to say that FM did not support chemotaxis. Positive chemotaxis toward FM had been expected (3, 25) and was observed by using both the Dunn and the under-agar assays. These results serve as an ideal positive control as they reflect the robustness of the methods used. However, analysis of *E. histolytica* responses to and from FM and CM, using maintenance medium without glucose (MM-1Gluc⁻) as a control, presents convincing evidence that both positive and negative chemotactic responses occur independently. Thus, results in our earlier assays and presumably those reported earlier (7) are the combined effect of both these processes. However, the negative response away from CM is in our hands stronger than the positive response toward FM.

A central result of our study is the observation that the commensal (nonpathogenic) *E. dispar* does not respond to the accumulation of metabolic products in the same way as the closely related *E. histolytica*. Both species (19) can inhabit the human intestine, but whereas the former is capable of causing life threatening intestinal and extraintestinal disease, the latter is not invasive (26). Biologically, the ability to invade and metastasize provides no obvious evolutionary advantage to *E. histolytica*. Cysts are never formed in tissues and tissue invasion is therefore a "dead-end street" for the parasite's life cycle. It is likely therefore that *E. histolytica* is an opportunistic pathogen, and that invasion occurs

“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 amoebae 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 amoebae 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 $275 \times g$ 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 *Dictyostelium* (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 μ l) 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, 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 2.5×10^6 amoebae/ml in MM-1, and 200 μ l of this cell suspension was added to the peripheral troughs. Plates were maintained at 36–37°C in a humidified atmosphere containing 5% CO₂ 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 $\approx$ 1 h before the assay, and cells were reincubated at 36°C. Cells were adjusted to 1×10^5 amoebae per ml in 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 $\times$ 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 chemotactic 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 Dunn 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.

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