SHORT COMMUNICATION

Chemotaxis as a Factor in Interactions between HeLa Cells and 
Salmonella typhimurium

By D. L. ULTMAN AND G. W. JONES*
Department of Microbiology and Immunology, University of Michigan, Ann Arbor,
Michigan 48109, U.S.A.

(Received 10 July 1981)

HeLa cells damaged by exposure to low pH released a diffusible attractant which greatly 
increased the collision frequency and hence the attachment of chemotactic Salmonella 
typhimurium. The attractant was tentatively identified as glycine. In contrast, when 
undamaged HeLa cells were used, no difference was found between a chemotactic parent 
strain and a non-chemotactic mutant in collision frequency or attachment. It is suggested that 
 factors increasing cell membrane permeability could attract S. typhimurium to host cells in vivo.

INTRODUCTION

Before internalization of Salmonella typhimurium by HeLa cells can occur, the bacteria 
must first collide with the HeLa cells, then become reversibly attached and subsequently 
irreversibly attached to the surface (Jones et al., 1981; Jones & Richardson, 1981). Conceivably, the frequency of collision and hence of attachment could be elevated if the 
salmonellae were attracted by taxins released by host cells; such attractants could be amino 
acids, sugars or tricarboxylic acid cycle intermediates (Mesibov & Adler, 1972; Melton et al., 
1978). To investigate this possibility, the chemotaxis of S. typhimurium towards HeLa cells 
was evaluated in terms of the frequency of contact between motile bacteria and HeLa cells.

METHODS

Bacteria and HeLa cells. The chemotactic Salmonella typhimurium strain S850 has been described previously 
(Jones et al., 1981). Non-chemotactic mutants produced by u.v. mutagenesis were isolated on a gradient column 
(Aswad & Koshland, 1975) with tryptone as the attractant. Bacteria were grown in Brain Heart Infusion broth 
(Difco) with shaking for 16 h at 37 °C and suspended in Hanks' Balanced Salt solution (Gibco, Grand Island, 
N.Y., U.S.A.) at pH 7.4 or pH 5.2 as specified. HeLa cells (strain S3; Microbiological Associates, Bethesda, Md. 
U.S.A.) were grown as non-confluent monolayers on 22 mm coverslips and prepared for use as described 
previously (Jones et al., 1981).

Collision frequencies and reversible and irreversible attachment. Washed HeLa cell monolayers on coverslips 
were overlaid with bacterial suspensions (1 x 10⁷ bacteria ml⁻¹); a depth of 0.15 mm was maintained by a 
supported overlying coverslip. Bacteria swam freely over and around the HeLa cells for more than 1 h at pH 7.4 
and pH 5.2. Observations were made by phase-contrast microscopy at room temperature for 30 min.

Collision frequency was defined as the number of bacteria that collided with a 20 μm length of HeLa cell edge 
during 1 min periods; collision frequencies were similar at all sites on the cell. A bacterium was said to reversibly 
attach to a HeLa cell if, after colliding with a HeLa cell, it remained in contact with the cell surface for a period 
longer than 1 s but could at any time be dislodged by fluid displacement (Jones et al., 1981) caused by tapping the 
coverslip. Irreversibly attached bacteria (Jones et al., 1981) were those resisting washing and fluid shear after a 30 
min incubation with HeLa cells at 37 °C.

0022-1287/82/0001-0049 $02.00 © 1982 SGM
Chemotaxis and chemotaxis inhibition. Chemotaxis towards HeLa cells (swarming) was induced with Hanks’ solution adjusted to pH 5.2 with HCl (see Results). Compounds (obtained from Sigma) used to inhibit swarming were dissolved in Hanks’ solution at pH 5.2. Concentrations up to a maximum of 100 mM, or the highest concentration that allowed normal bacterial motility (0.1 mM-glutamate, 1.5 mM-cysteine, 10 mM-valine, 1 mM-tryptophan and 5 mM-galactose) or which produced near saturated solutions (10 mM-phenylalanine, 20 mM-leucine and 1 mM-tyrosine) were used. The entire monolayer was observed for 30 min and the presence or absence of swarming with and without these compounds was recorded.

In the capillary tube tests (Adler, 1973), the chemotaxis medium was Hanks’ solution at pH 7.4 or pH 5.2, with 0.1% (v/v) Triton X-100 (Sigma) added to prevent bacteria from sticking to the capillary tube (Triton X-100 had no effect on bacterial motility or on chemotaxis). Tests were done in triplicate and incubated for 1 h at 37 °C with suspensions of 1 x 10⁷ bacteria ml⁻¹. The results were expressed as the number (viable count) of bacteria in capillary tubes with attractant present minus the number of bacteria in capillary tubes without attractant. At pH 7.4 and pH 5.2 the number of bacteria in capillary tubes containing tryptone as attractant was about 1000 and with Hanks’ solution alone was about 50. Chemotaxis inhibition (Mesibov & Adler, 1972) was expressed as the percentage reduction of chemotaxis with inhibitor present compared with the control without inhibitor.

RESULTS AND DISCUSSION

At pH 7.4, chemotactic bacteria of strain S850 collided with HeLa cells and reversibly attached perpendicular to the surface. The unattached pole which was nearer the observer rotated clockwise suggesting that the flagellar bundle continued to rotate (Berg, 1974) and hence imparted a forward thrust on the bacterial cell. Reversible attachment occurred equally well in 160 mM-sucrose indicating that it was maintained by the bacterium’s forward motile force and not by long range London-van der Waals’ forces of attraction (Jones et al., 1981). In this low ionic strength medium the distance of the bacterium from the HeLa cell is too great for these attractive forces to be effective (Marshall et al., 1971). As reported previously, reversible attachment preceded irreversible attachment (that was sensitive to changes in ionic strength) and this, in turn, led to internalization (Jones et al., 1981). It should be noted, however, that a form of irreversible attachment that was insensitive to changes in ionic strength was also observed; this was neither preceded by reversible attachment nor followed by internalization. It appeared to be caused by the irreversible entanglement of the bacteria in the long microvilli of the HeLa cell surface (Kihlström & Latkovic, 1978).

At pH 7.4, the chemotactic parent strain and a non-tumbling, non-chemotactic mutant of it showed statistically indistinguishable collision frequencies and rates of both reversible and irreversible attachment. Collision frequencies (± standard deviation) were 21.1 ± 4.7 min⁻¹ (20 μm)⁻¹ and 22.0 ± 4.6 min⁻¹ (20 μm)⁻¹ for parent and mutant, respectively. Reversible attachment occurred at rates of 3 per 10 collisions and irreversible attachment at 4 per 1000 collisions for both strains. At pH 5.2, however, only the chemotactic bacteria were seen to form swarms around HeLa cells. Chemotactic bacteria leaving the vicinity of an attractive HeLa cell tumbled approximately 10 μm from the surface. Swarms developed as a proportion of these bacteria moved back towards the HeLa cell upon regaining their swimming mode. The tumbling of the bacteria defined the outer edge of the swarm and the HeLa cell defined its inner border. Swarming increased the collision frequency threefold.

At pH 5.2, the HeLa cells showed visible membrane damage (blebs) after about 15 min. Swarms often formed around HeLa cells before blebs had appeared and always while the HeLa cells were still impermeable to Trypan blue. Swarms were of different densities and lasted for 5–20 min before becoming less dense and eventually dispersing. Fresh bacterial suspension added before a swarm began to disperse formed a swarm of the same density as the previous swarm suggesting that swarm density was controlled by the HeLa cell. It was postulated that the release of a low molecular weight attractant by the HeLa cells created chemotactic gradients extending about 10 μm into the surrounding medium and that the swarms dispersed when the bacterial chemoreceptors became saturated (Adler, 1973).

In order to characterize this HeLa cell attractant, known chemoattractants were dispersed uniformly throughout the bacterial suspension to saturate bacterial chemoreceptors
Short communication

Table 1. Inhibition of chemotaxis and swarm formation

The percentage inhibition of chemotaxis of *S. typhimurium* strain S850 to aspartate, asparagine, alanine, serine or glycine was measured in the capillary tube test to which inhibitors were added at the concentrations shown. The means of three determinations are given (the variation for the three determinations was less than 10%); 100% inhibition represents the background level of approximately 50 bacteria per capillary tube and 0% inhibition represents a normal chemotactic response of 500 or more bacteria per capillary tube.

The inhibition of swarming around HeLa cells was measured in the presence of the same concentrations of inhibitors (+, inhibition; −, no inhibition).

<table>
<thead>
<tr>
<th>Inhibitor (conc)</th>
<th>L-Aspartate (1 mM)</th>
<th>L-Asparagine (10 mM)</th>
<th>L-Alanine (100 mM)</th>
<th>L-Serine (100 mM)</th>
<th>L-Glycine (100 mM)</th>
<th>Inhibition of swarm formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Aspartate (1 mM)</td>
<td>100</td>
<td>97</td>
<td>87</td>
<td>0</td>
<td>75</td>
<td>+</td>
</tr>
<tr>
<td>L-Asparagine (10 mM)</td>
<td>100</td>
<td>100</td>
<td>88</td>
<td>0</td>
<td>60</td>
<td>+</td>
</tr>
<tr>
<td>L-Alanine (100 mM)</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>68</td>
<td>+</td>
</tr>
<tr>
<td>L-Serine (100 mM)</td>
<td>66</td>
<td>58</td>
<td>91</td>
<td>100</td>
<td>58</td>
<td>+</td>
</tr>
<tr>
<td>L-Glycine (100 mM)</td>
<td>95</td>
<td>95</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>+</td>
</tr>
<tr>
<td>L-Tyrosine (1 mM)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>−</td>
</tr>
<tr>
<td>Citrate (10 mM)</td>
<td>63</td>
<td>100</td>
<td>71</td>
<td>100</td>
<td>0</td>
<td>−</td>
</tr>
<tr>
<td>Maltose (100 mM)</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>−</td>
</tr>
</tbody>
</table>

(Mesibov & Adler, 1972). If the test substances and HeLa cell attractant shared the same chemoreceptor, the high concentrations of the substance should saturate the receptor and block response to the attractant gradient, i.e. inhibit swarming. Of the 20 amino acids examined, only aspartate, asparagine, alanine, serine and glycine inhibited swarming. Swarming was not inhibited by maltose, fructose, galactose or citrate.

All substances tested for swarm inhibition were tested also as chemotactic attractants at pH 5.2 in capillary tube tests. Citrate, aspartate, asparagine, alanine, serine, glycine and maltose were most strongly attractive with responses at least 10 times the control value of 50 bacteria per capillary tube. Since aspartate, asparagine, alanine, serine and glycine were all both attractive and able to block swarming, one or more were considered to be the HeLa cell attractant. These five amino acids were used as attractants in capillary tube inhibition tests. In Table 1 are given the means of three determinations of the percentage inhibition of chemotaxis. It can be seen that only glycine resembles the HeLa cell attractant. The attractant, therefore, may either share the glycine chemoreceptor or be glycine itself.

At first sight the chemotactic response of *S. typhimurium* to HeLa cells at pH 5.2 may appear artificial. The bacteria, however, did exhibit normal chemotactic behaviour in the acid medium and it seemed that the pH served simply to cause and maintain the leakage of intracellular substances from the HeLa cell. It is probable, therefore, that any condition that reduces the integrity of host cell membranes could invoke a similar chemotactic response. Glycine may not always be the attractant released by stressed cells, but there is some evidence that *S. typhimurium* are attracted to debilitated cells *in vivo*. Gianella *et al.* (1973), working with rabbit ileal loops, found that *S. typhimurium* attached preferentially to the villus tips where both invasion and multiplication occurred. Dead and dying epithelial cells are sloughed from the villus tips and chemotaxis to these dying cells could increase bacterial contact with the host mucosa to a threshold needed for infection (Freter *et al.*, 1979).

This work was supported by Biomedical Research Support Grant RR05383 awarded to The University of Michigan by the National Institutes of Health, and a University of Michigan Student Research Fellowship to D.L.U.


