SHORT COMMUNICATION

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

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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 (1×10^7 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.

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 1×10^5 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 ($\pm$ standard deviation) were $21.1 \pm 4.7 \text{ min}^{-1}$ ($20 \mu\text{m}$) $^{-1}$ and $22.0 \pm 4.6 \text{ min}^{-1}$ ($20 \mu\text{m}$) $^{-1}$ 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

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).

Inhibitor (concn)	Percentage inhibition of chemotaxis towards attractants					Inhibition of swarm formation
	L-Aspartate (1 mM)	L-Asparagine (10 mM)	L-Alanine (100 mM)	L-Serine (100 mM)	L-Glycine (100 mM)	
L-Aspartate (1 mM)	100	97	87	0	75	+
L-Asparagine (10 mM)	100	100	88	0	60	+
L-Alanine (100 mM)	0	0	100	0	68	+
L-Serine (100 mM)	66	58	91	100	58	+
L-Glycine (100 mM)	95	95	100	0	100	+
L-Tyrosine (1 mM)	0	0	0	0	0	—
Citrate (10 mM)	63	100	71	100	0	—
Maltose (100 mM)	0	50	0	0	0	—

(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).

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