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Locusts as models for studying brain diseases and behaviour

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Abstract: Two examples illustrate the use of locusts as models for the study of brain disease and behaviour. First, immune challenge induces sickness behaviour in locusts: immune challenge with laminarin induces anorexia, paralleling phenomena seen in other animals including mammals. Laminarin-induced anorexia in locusts can be blocked by Mianserin, suggesting the involvement of serotonergic receptors. Second, the blood-brain barrier in locusts can be used to study virulence factors in *Escherichia coli* K1, one of the causative agents of bacterial meningitis in humans: two of the known virulence determinants in mammals, OmpA and CNF1, are also essential for invasion of the locust CNS.

Keywords: *Escherichia coli*, pathogenicity, blood-brain barrier, locusts, OmpA, CNF1, laminarin, sickness behaviour, illness-induced anorexia

INTRODUCTION

Virulence factors for opportunistic bacterial and fungal pathogens of humans can often be modelled successfully using insects [1]. Here two examples are described of the use of locusts to study brain infection and behaviour relevant to the human condition: the crossing of the blood-brain barrier by human pathogens can be studied in the locust, without the legislative restrictions and ethical concerns associated with work on mammals. *Escherichia coli* sepsis and meningitis are among leading causes of neonatal bacterial infections, but only a handful of virulence determinants for *E. coli* K1 invasion of the mammalian central nervous system (CNS) have been identified. Here it is shown that at least two of these are also important in determining pathogenicity and invasion

of the brain in locusts. A second example concerns sickness behaviour, which includes behavioural and physiological changes that develop in animals during infection: insects manifest this as, for example, behavioural fever and anorexia [2]. It is likely that in insects, as in mammals, sickness behaviour is cytokine-driven, although only a few insect cytokines are known. Here, the phenomenon of immunogen-induced anorexia in locusts is described, and a parallel drawn with sickness-induced anorexia in mammals.

MATERIALS AND METHODS

Locusts

Adult male African migratory locusts, *Locusta migratoria* L., from 15-30 days old were reared at Birkbeck and fed on bran, wheat seedlings, and fresh grass [3].

Bacterial strains and culture conditions

Escherichia coli K1 strain RS218 (O18:K1:H7) is a cerebrospinal fluid isolate from a neonate with meningitis. Two isogenic gene-deletion mutants of K1 were used: Δ*ompA* (outer membrane protein A mutant) [4]; Δ*cnf1* (cytotoxic necrotizing factor-1 mutant) [5]; and *Escherichia coli* K12 strain, HB101 (a non-invasive isolate) was used as a negative control.

Determination of induced mortality and invasion of the CNS

To determine the virulence of *E. coli*, locusts were injected individually with 20 μl of broth containing $2x10^6$ cells of the strain under test. Six replicates, each of ten injected locusts were set up and mortality recorded every 24 h. To determine invasion of the CNS, brains dissected from locusts, infected 24 h previously, were washed in PBS, and incubated with gentamicin (100 μg/ml final concentration) at 37 °C for 60 min to kill extracellular bacteria. The brains were then washed thoroughly to remove gentamicin, and lysed in 0.5% SDS before being plated on nutrient agar plates.

Feeding assays

Locusts were observed to feed on fresh wheat seedlings and then placed without food for 2 h before being injected with 20 μl of either Ringer or the β-1,3 glucan immunogen, laminarin (10 μg in 20 ul of Ringer). After 10 min, locusts were placed on fresh wheat seedlings and observed individually every minute for 1 h to record whether they were feeding or not. In some experiments,

10 μl of a solution (2 x 10^{-2} M in sterile saline) of the serotonin receptor blocker Mianserin (10^{-3}M final estimated concentration in the haemolymph), were injected 10 min before the laminarin, and in other experiments 10 μl of a solution of serotonin (2 x 10^{-2} M in sterile saline) alone was injected (10^{-3} M estimated final concentration in the haemolymph) 10 min before observing the feeding behaviour.

RESULTS

Mortality and invasion of the brain after injection of *E. coli* K1 and its gene-deletion mutants

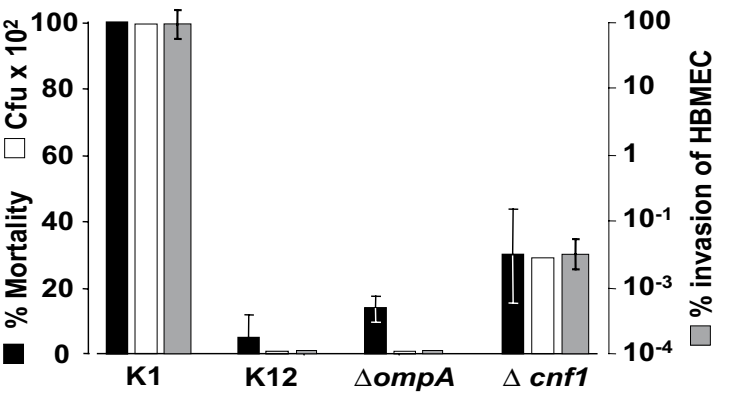

Figure 1. Mortality caused by injection of *Escherichia coli* K1 into locusts, and the ability to invade locust brain. *E. coli* K1 caused >90% mortality (solid bars) within 72 h of injection, whereas injections of K12 resulted in <5% mortality. The deletion mutants of *E. coli* K1 were both attenuated significantly *(P<0.05 in both cases: Student's t-test)* in their virulence compared with the parent strain. These differences were reflected in the numbers of bacteria found in the brains of locusts (open bars) 24 h after injection: The Δ*ompA* and K12 strains were not found in significant numbers in the brain tissue, whereas K1 was present in large numbers, and Δ*cnf1* numbers were *c*. 30% of that of the parent strain. For comparison, data from Khan *et al*. (2003) showing the ability of these strains to invade human brain microvascular endothelial cells (HBMEC) *in vitro* (cross-hatched bars) are plotted alongside the present data. Values are mean ± SE for at least 10 observations.

Of the locusts injected with K1, >95% died within 72 h, whereas injections of K12 caused <5% mortality (Figure 1). Mortality in CNF1 or OmpA mutant-injection was significantly less than that induced by K1 (Figure 1). The average number of live bacteria recovered from each gentamicin-treated brain was *c.*10,000 CFU for K1, whereas the *cnf1*-mutant was present in low numbers, and K12 and the *ompA*-deletion mutant were not found in the brains of locusts (Figure 1).

Laminarin-induced anorexia

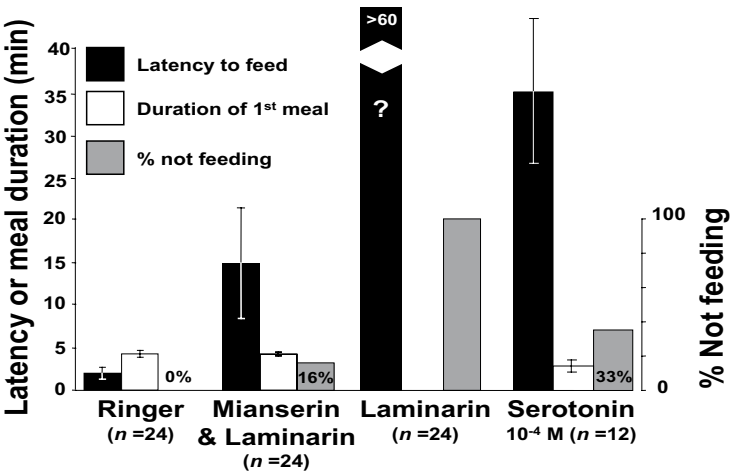

Figure 2. The feeding behaviour of adult male locusts assessed as the latency to feed and the duration of the first meal. Injection of an optimal amount of laminarin (>10 ug) completely prevented feeding for at least 1 h (i.e. 100% not feeding, hatched bars), so no estimate of latency (solid bars) can be given, and meal duration (open bars) was zero, with 100% of the locusts not feeding. Injection of Mianserin 10 min before the laminarin prevented this anorexia and most of these insects ate but showed a significantly increased latency to feed (P<0.05: Student's t-test) with meal duration similar to that in Ringer-injected locusts. Injection of serotonin alone elicited an anorectic response in *c*. 33% of the locusts, while those that did eat showed a marked increased in latency to feed (P<0.01) without any apparent effect on meal duration. Values are mean ± SE.

Injection of 10 μg of laminarin produced an immediate anorectic response lasting at least 60 min: laminarin-injected locusts perched on the vertical blades

of wheat seedlings, but showed no attempt to taste (palpate) or eat the leaf. Low but near-optimal doses of laminarin (5 μg) did not stop most locusts from feeding, but increased the latency to feed while not affecting meal duration (Figure 2). When locusts that received an optimal dose of laminarin (10 μg) administered 10 min after being injected with mianserin, many of them ate, but with a greater latency than Ringer-injected locusts and with 'normal' meal duration. Locusts injected only with serotonin had an increased latency to feed but meal duration was unaffected (Figure 2). Injection of octopamine had no noticeable effect on feeding behaviour (data not shown).

DISCUSSION

Crossing the blood-brain barrier

In vertebrates and insects, the blood-brain barrier is a physical limit to the penetration of the brain by circulating bacteria, or large molecules, so in insects as in mammals, bacteria must cross the blood-brain barrier via a transcellular route. The results shown here suggest that at the molecular level there are similarities between the mechanisms by which *E. coli* K1 invades the CNS of mammals and of locusts, especially, for example, in the involvement of OmpA in host cell recognition, and Cnf1 in inducing phagocytocis of the bacteria. The parallels between mortality and invasion of the locust brain, and the capacity of the same strains to invade human endothelial cells [6] are remarkable, as is illustrated by the comparison in Figure 1.

Laminarin-induced sickness behaviour

Locusts display pronounced anorexia when their immune system is challenged by the injection of laminarin, and it seems likely that serotonin is involved in this phenomenon, but the site of its production and release is unknown. Whether cytokines are involved in the locust anorexia described here also remains to be determined but this seems likely [7] and is being investigated currently. Intriguingly, there is a similar interaction between mianserin and serotonin on feeding behaviour in rats [8]. The increased latency to feed seen in locusts injected with sub-optimal doses of laminarin suggests that this aspect of sickness behaviour may result from reduced sensory input as occurs normally towards the end of a meal and is signalled by the release of the locust CRF-like diuretic hormone [9]. It is intriguing that sickness-induced anorexia could involve serotonin which also acts as a diuretic hormone in other insects [10].

A whole-organism approach to the study of disease physiology is essential to gaining a full understanding of host-pathogens interactions. While mammalian models are immediately more relevant, the locust model described here could be a valuable tool to discriminate molecules participating from both sides of the host-bacterial interaction, and generate potential leads to be tested subsequently in mammalian systems.

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