Relationship Between Clinical Signs and Transmission of an Infectious Disease and the Implications for Control

Bryan Charleston,[1]* Bartlomies M. Bankowski,[1] Simon Gubbins,[1] Margo E. Chase-Topping,[2] David Schley,[1] Richard Howey,[2] Paul V. Barnett,[1] Debi Gibson,[1] Nicholas D. Juleff,[1] Mark E. J. Woolhouse[2]

Control of many infectious diseases relies on the detection of clinical cases and the isolation, removal, or treatment of cases and their contacts. The success of such "reactive" strategies is influenced by the fraction of transmission occurring before signs appear. We performed experimental studies of foot-and-mouth disease transmission in cattle and estimated this fraction at less than half the value expected from detecting virus in body fluids, the standard proxy measure of infectiousness. This is because the infectious period is shorter (mean 1.7 days) than currently realized, and animals are not infectious until, on average, 0.5 days after clinical signs appear. These results imply that controversial preemptive control measures may be unnecessary; instead, efforts should be directed at early detection of infection and rapid intervention.

Strategies to control the spread of many infectious diseases rely wholly or partly on reactive measures implemented upon the detection of a clinical case. Examples include human influenza, diphtheria, pertussis, pneumonic plague, severe acute respiratory syndrome (SARS), and viral haemorrhagic fevers, as well as major animal diseases such as classical swine fever, foot-and-mouth disease, highly pathogenic avian influenza, and swine vesicular disease (*1*). For these diseases, once a clinical case is detected the affected individual may be treated or isolated or (for livestock diseases) culled with the aim of limiting opportunities for further transmission. In some circumstances, prophylaxis, quarantine, or culling of at-risk individuals (usually those in close physical proximity to a case or identified by contact tracing) is also implemented. Such measures are often contentious (*2*, *3*) and are defended on the grounds of their perceived contribution to reducing transmission rates and so protecting public or animal health.

The success of reactive disease control strategies has previously been shown to depend on the timing of the onset of infectiousness relative to the onset of detectable clinical signs (*4*). The key variable is θ, the fraction of transmission that occurs during the overlap of the incubation period (time from exposure to onset of signs) and the infectious period. If θ is small, then reactive control targeted only at clinical cases may be effective. For moderate values of θ (or for low values of θ if there is a substantial delay implementing control measures), additionally targeting at-risk individuals may be warranted. However, if θ is too large—if most transmission occurs before disease is apparent (such as in the case of HIV/AIDS)—reactive control measures will be ineffective. Three successful disease eradication campaigns—smallpox, SARS, and rinderpest—were facilitated by low θ values (*4*, *5*).

The means and distributions of incubation, latent, and infectious periods are key determinants of θ and have been estimated for many infectious diseases (*6*–*9*), but the value of θ also depends on their joint distributions, which are less well studied. Here, we report how we quantified these distributions for foot-and-mouth disease (FMD) in cattle and assess the implications of the results for the design of control strategies. We go on to consider the relevance of the findings to other infectious diseases.

Foot-and-mouth disease virus (FMDV) is a RNA virus of the Picornaviridae family (a group containing a number of animal and human pathogens) that naturally infects cattle and other livestock species, causing an acute illness characterized by fever, nasal discharge, and lesions on the tongue and/or feet. It is one of the world's most important animal pathogens, responsible for huge global losses to livestock production and trade, as well as frequent and highly disruptive large-scale epidemics (*10*).

We carried out an experimental study of direct transmission of FMDV between pairs of cattle kept indoors in close proximity for 8 hours, with room temperature, humidity, and air circulation optimized during pilot studies for transmission to occur. Briefly, eight "source" cows were successfully exposed to infection by direct contact with cattle injected with the FMDV serotype O isolate circulating in the UK in 2001, and transmissions to naïve cows were attempted at 2-day intervals post exposure (*11*). This design allowed us to study individual transmission events occurring at specific time points after exposure, in contrast to previous studies that estimated net FMDV transmission rates for small groups of animals in contact for extended periods (*12*, *13*).

There were only eight successful transmissions (from seven of the cows) in 28 attempts, even though we detected FMDV in blood (viraemia), nasal fluid (NF), and/or oesophageal-pharyngeal fluid (OPF) on all but one occasion (table S1). We quantified a set of 23 virological, immunological, and clinical variables for each of the source cows (table S2). From these, we created composite variables using the data reduction method nonmetric multidimensional scaling (NMS) (*11*). NMS score was strongly associated with infectiousness ($P = 0.0002$) (Fig. 1A and table S3). Moreover, NMS axis 1 and 2 together provided an informative representation of the sequence of events that occur during FMDV infection and, crucially, how these relate to infectiousness (Fig. 1B). We depict these in relation to a reference time point, day P, which corresponds to the day of peak NMS axis 1 score for each infected cow. There is an initial quiescent phase lasting 1 to 4 days; previous studies suggest the variation is due to differences in the infectious dose received (*14*). Day P-1 is marked by the first appearance of high levels of viraemia. On day P, there is a rapid cascade of events including the detection of live virus in nasal fluid and the onset of clinical signs and a type-1 interferon response, all of which are heavily weighted components of NMS axis 1 (fig. S1). On day P+1, there is a decrease in the amount of detectable virus because a sharp peak in the level of type-1 interferon prevents virus from infecting additional epithelial cells where most replication occurs (*15*), although some clinical signs persist. From day P+2 onwards, only low levels of virus and interferon are detectable, but FMDV-specific antibodies are present. Six out of eight successful transmissions occurred on day P, a highly significant association (exact $P = 0.0064$). These results suggest that conditions promoting transmission exist for only a brief period and clearly show that infectiousness is a complex phenomenon related not just to virus dynamics but also to host responses and clinical signs, which is consistent with a general but rarely tested expectation that disease signs may be functionally linked to infectiousness (*16*).

The experimental data allowed us to make formal estimates of the infectious period, the latent period, and the incubation period; clinical signs were defined here as any visible lesions or body temperature above 39.5°C. We did this using a Bayesian framework that allowed us to draw inferences about the unobserved latent and infectious periods according to the outcome of each transmission attempt. The mean latent and incubation periods were estimated to be 4.6 days [95% credible interval (CI): 3.1 to 7.2 days] and 4.1 days (2.9 to 5.9 days), respectively (Fig. 2, A and B). These variables were significantly correlated (correlation $\rho = 0.77$, 95% CI: 0.30 to 0.96) (Fig. 2C), and the mean infectious period was short: 1.7 days (0.3 to 4.8 days) (Fig. 2D). Both of these results are consistent with the NMS analysis. The statistical model was a good description of the transmission data (Fig. 2E).

Previous estimates of the latent and infectious periods for FMDV have used indicators such as the detection of virus in blood, NF, or OPF as

[1]Institute for Animal Health, Pirbright Laboratory, Ash Road, Woking, Surrey GU24 0NF, UK. [2]Centre for Immunity, Infection and Evolution, University of Edinburgh, Ashworth Laboratories, Kings Buildings, West Mains Road, Edinburgh EH9 3JT, UK.

*To whom correspondence should be addressed. E-mail: bryan.charleston@bbsrc.ac.uk

proxy measures of infectiousness (*13*) rather than directly demonstrating transmission to another animal. Using these measures from our experimental data gave significantly shorter estimates of the mean latent period (0.5 to 2.7 days) (Fig. 2A) and much longer estimates of the mean infectious period (4.2 to 8.2 days) (Fig. 2D). These estimates are very similar to the results of a recently published meta-analysis of data on FMDV serotype O in cattle (*17*). Additionally, when we used proxy measures of infectiousness the latent period appeared longer than the incubation period [whereas the transmission data suggested it was shorter (Fig. 2, A and 2)], and the correlation between latent and incubation periods was weaker or entirely absent (Fig. 2C). Similar proxies for infectiousness are routinely used in studies of not just FMDV but many other human and animal pathogens (*6*–*8*).

Extending previous analyses (*4*) to allow for jointly distributed latent and incubation periods, the proportion of transmission occurring before the onset of clinical signs is given by

$$\theta = \frac{\int_0^\infty \int_0^\tau \left\{ \int_\tau^\infty f(E,C)\mathrm{d}C \right\} \left\{ \int_{\tau-E}^\infty g(I)\mathrm{d}I \right\} \mathrm{d}E\,\mathrm{d}\tau}{\int_0^\infty \int_0^\tau f_E(E) \left\{ \int_{\tau-E}^\infty g(I)\mathrm{d}I \right\} \mathrm{d}E\,\mathrm{d}\tau} \quad (1)$$

where $f(E,C)$ is the joint probability distribution function (PDF) for the latent and incubation periods, $f_E(E)$ is the marginal PDF for the latent period, and $g(I)$ is the PDF for the infectious period [see (*11*) for derivation of this expression]. As shown in Fig. 3A, with parameters based on virus isolation in blood, NF, or OPF, the median estimate of θ was 0.43, 0.27, or 0.44, respectively (see Fig. 3A for PDFs), with the possibility that a cow could be infectious for several days before showing clinical signs. Using a direct measure of infectiousness, the median estimate of θ was only 0.13 (Fig. 3A), and an animal that was infectious before clinical onset would most likely be so for only a few hours.

Sensitivity analysis of Eq. 1 indicates that the effects reported here for FMD could potentially apply to any acute infectious disease (*11*). The crucial factor is whether the variance of the timing of the onset of infectiousness relative to signs is large in comparison with the infectious period. For human influenza, for example, the value of θ has been reported as 0.3 to 0.5 (*4*), yet several authors have suggested, on the basis of observational data, that it could be much lower (*8*, *9*, *18*). Resolving this debate for influenza or any other acute infection will require experimen-

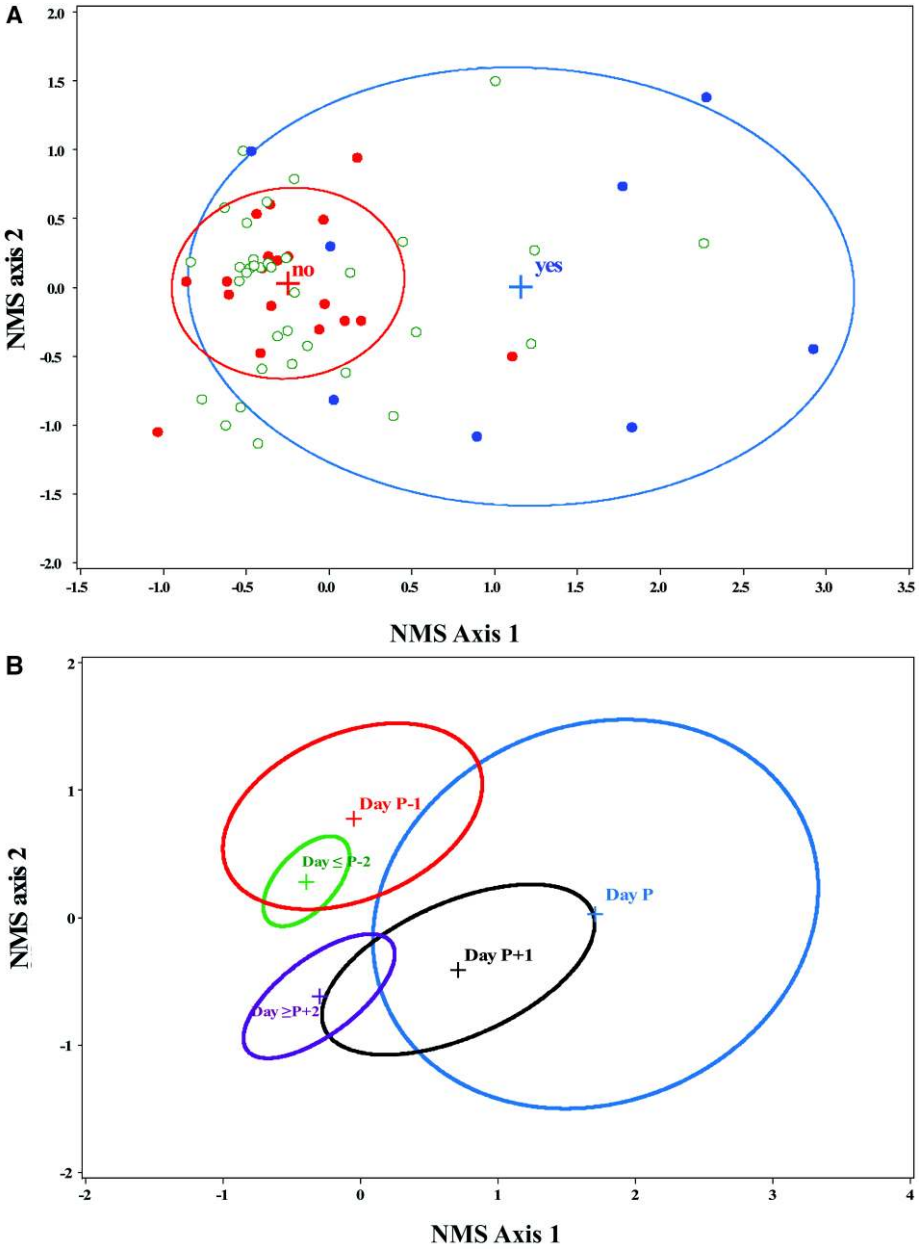

Fig. 1. NMS ordination of transmission data. The NMS final solution was two dimensional and explained 86.1% of the variation in FMD transmission success. Correlations between variables used (table S2) and NMS scores are shown in fig. S1. (**A**) Blue circles represent days when transmission occurred, red circles when no transmission occurred, and green open circles when transmission was not attempted. Ovals indicate the mean ±1 SD bivariate interval for successful and unsuccessful transmission attempts only. (**B**) Ovals indicate the mean ±1 SD bivariate interval for each day, where day P is the day of peak NMS axis 1 score. Days ≥P+2 and ≤P–2 have been grouped.

tal and/or epidemiological studies of transmission in natural hosts designed to quantify transmission rates at different times after exposure.

The combined effect of the differences between our findings and previous work based on proxy measures of infectiousness is that cattle infected with FMDV are substantially less likely to be infectious before showing clinical signs than is currently realized, implying that the need for reactive control measures targeted at "at-risk" farms, notably pre-emptive culling (*19*), has been overestimated. The likelihood of transmission is dramatically decreased if control can be implemented just 24 hours earlier; this effect is greatly underestimated if proxy measures of infectiousness are used (Fig. 3B). This result provides strong support for investment in the development of practical tools for preclinical diagnosis (*20*, *21*) because the onset of detectable viraemia typically occurs at ≥1 day before infected cows become infectious and/or show clinical signs (Fig. 1B and table S1). The same argument also suggests that the penalties for delayed detection of cases and/or implementation of control are even greater than is currently realized (Fig. 3B). Also, for the future our results suggest that prophylaxis, such as antiviral therapy, targeted at contacts could be used preclinically with greater confidence of preventing transmission. Lastly, we suggest that there is a need for more robust empirical evidence on relationships between clinical signs and infectiousness to underpin policy, not only for FMDV but also other acute infections for which reactive

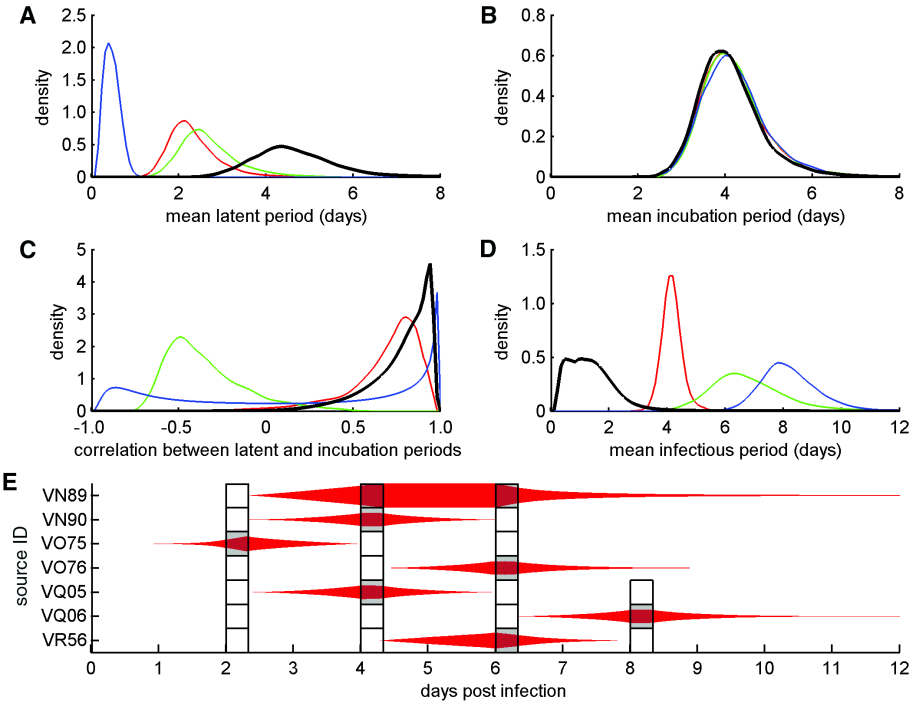

Fig. 2. Bayesian analysis of FMDV transmission data. (**A** to **D**) Marginal posterior densities for the mean duration (in days) of the (A) latent and (B) incubation periods, (C) the correlation between the latent and incubation periods, and (D) the infectious period. Results for the analysis based on transmission attempt outcome only (black lines) were compared with results for virus isolation from NF (green lines), blood (red lines), or OPF (blue lines). There were significant ($P < 0.05$) differences for latent period (blood and OPF), latent period minus incubation period (blood and OPF), their correlation (NF), and infectious period (NF, blood, and OPF). (**E**) Posterior estimates for the (unobserved) latent and infectious periods in relation to the experimental transmission attempts (indicated by boxes marked gray if the attempt was successful and white if it was not). The thickness of the red shapes indicates the proportion of Markov chain Monte Carlo samples for which an animal was infectious at that time (with the symbol occupying the full width of the box if it was infectious for all samples). Cow VR57 was excluded from these analyses because although infected, it was apparently never infectious.

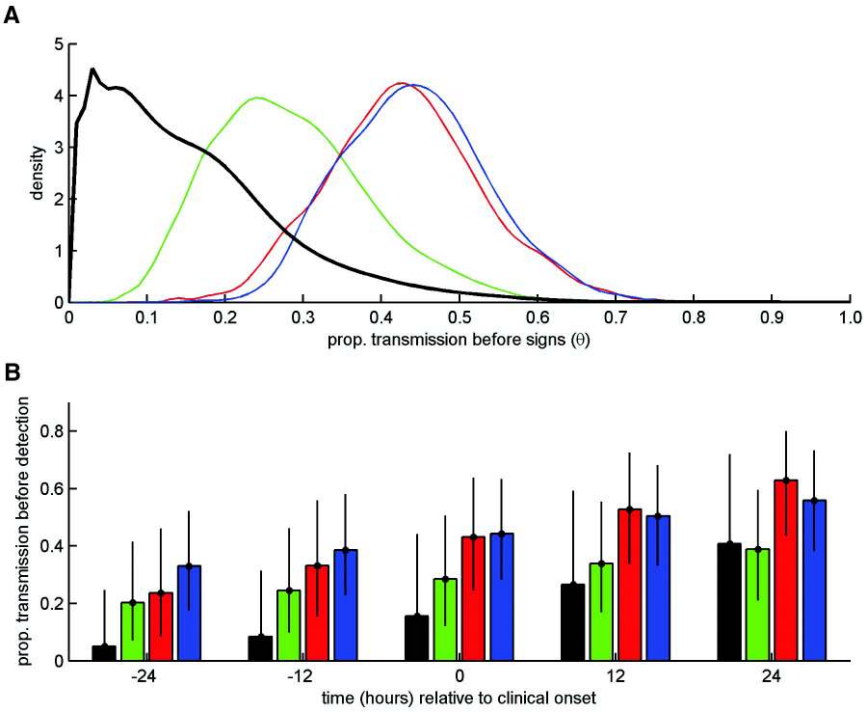

Fig. 3. Implications of results for detection and control of FMDV. (**A**) Marginal posterior density for the proportion of transmission that occurs before the onset of clinical signs, θ. (**B**) Posterior means (bars) and 95% credible intervals (error bars) for the proportion of transmission that occurs before detection assuming infected animals are detected at −24, −12, 0, +12, or +24 hours relative to the onset of clinical signs. In each plot, results are shown for the analysis based on transmission attempt outcome only (black) and virus isolation from NF (green), blood (red), or OPF (blue).

measures are an important component of control strategies.

References and Notes

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Supporting Online Material

www.sciencemag.org/cgi/content/full/332/6030/726/DC1
Materials and Methods
SOM Text
Figs. S1 and S2
Tables S1 to S5
References

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Neuronal GPCR Controls Innate Immunity by Regulating Noncanonical Unfolded Protein Response Genes

Jingru Sun, Varsha Singh, Rie Kajino-Sakamoto, Alejandro Aballay*

The unfolded protein response (UPR), which is activated when unfolded or misfolded proteins accumulate in the endoplasmic reticulum, has been implicated in the normal physiology of immune defense and in several human diseases, including diabetes, cancer, neurodegenerative disease, and inflammatory disease. In this study, we found that the nervous system controlled the activity of a noncanonical UPR pathway required for innate immunity in *Caenorhabditis elegans*. OCTR-1, a putative octopamine G protein–coupled catecholamine receptor (GPCR, G protein–coupled receptor), functioned in sensory neurons designated ASH and ASI to actively suppress innate immune responses by down-regulating the expression of noncanonical UPR genes *pqn/abu* in nonneuronal tissues. Our findings suggest a molecular mechanism by which the nervous system may sense inflammatory responses and respond by controlling stress-response pathways at the organismal level.

Endoplasmic reticulum (ER) stress has been linked to several human diseases, including diabetes, cancer, neurodegenerative disease, and inflammatory disease (*1*–*3*). The ER has developed specific signaling pathways, known as the unfolded protein response (UPR), to cope with ER stress and restore ER homeostasis. Recent studies indicate that increased demand on protein folding in the ER, which may occur during bacterial infections, must be alleviated by UPR pathways for a complete immune response to be mounted (*4*–*8*).

We took advantage of the simple nervous and immune systems of the nematode *Caenorhabditis elegans* to investigate the role of the nervous system in the organismal control of pathways involved in innate immune responses. Three sensory neurons (AQR, PQR, and URX) are known to regulate resistance to pathogen infections by controlling the activation of a p38 mitogen-activated protein kinase (MAPK) pathway and the *C. elegans* avoidance to certain pathogens (*9*, *10*). In addition, a range of chemosensory neurons that penetrate the cuticle and directly detect and respond to different environmental cues have the potential to control innate immune responses.

To elucidate the molecular mechanism by which these neurons regulate innate immunity in response to pathogen infection, we first determined the susceptibility to the human opportunistic pathogen *Pseudomonas aeruginosa* of *octr-1(ok371)* animals, which lack a G protein–coupled catecholamine receptor normally expressed in the cilia of neurons located in sensory openings, including ASH, ASI, AIY, and dopaminergic ADE/CEP neurons (*11*, *12*). *octr-1(ok371)* animals exhibited enhanced resistance to killing by *P. aeruginosa* (Fig. 1A), which suggests that the loss of OCTR-1 signaling increases the general immune function of the nematodes. We observed no difference in survival between *octr-1(ok371)* and wild-type (WT) animals that were fed heat-killed *P. aeruginosa* or *Escherichia coli* (Fig. 1B and fig. S1). Thus, *octr-1* mutation affects the immune response to living pathogenic bacteria without altering the basic life span of the nematodes.

Because pathogen avoidance is part of the *C. elegans* defense response to *P. aeruginosa* (*9*), we examined the susceptibility to *P. aeruginosa*–mediated killing of *octr-1(ok371)* animals on agar plates that were completely covered in bacteria, a condition that eliminates pathogen avoidance. *octr-1(ok371)* animals died at a slower rate than did WT animals (Fig. 1C), indicating that pathogen avoidance does not play a role in *octr-1(ok371)*–enhanced resistance to *P. aeruginosa.* In addition, the magnitude of pathogen avoidance of *octr-1(ok371)* and WT animals was similar (Fig. 1D). The enhanced resistance of *octr-1(ok371)* to *S. enterica* (fig. S2), a pathogen that does not elicit an avoidance behavior (*13*), further supports the function of OCTR-1 in the regulation of immune responses.

Certain mutants resistant to pathogen infection exhibit resistance to pathogen accumulation (*14*). Therefore, we examined whether *octr-1* mutation affected bacterial accumulation in the intestine. Compared to WT animals, *octr-1(ok371)* animals exhibited a similar accumulation pattern of *P. aeruginosa*/green fluorescent protein (GFP) or *S. enterica*/GFP (Fig. 1E and fig. S3). In addition, the number of bacterial cells in *octr-1(ok371)* animals was similar to that in WT animals (Fig. 1F and fig. S4). Thus, the bacterial load to which the animals were exposed was comparable, and reduced bacterial accumulation levels in the intestine did not contribute to the enhanced immunity of *octr-1(ok371)* animals, indicating that *octr-1(ok371)* animals exhibit enhanced endurance to *P. aeruginosa* infection.

To provide insights into the mechanism underlying the enhanced immunity of *octr-1(ok371)* ani-

Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC 27705, USA.

*To whom correspondence should be addressed. E-mail: a.aballay@duke.edu