

9. Leith, N. J. & Barrett, R. J. Amphetamine and the reward system: evidence for tolerance and post-drug depression. *Psychopharmacologia* **46**, 19–25 (1976).
10. Markou, A. & Koob, G. F. Postcocaine anhedonia: an animal model of cocaine withdrawal. *Neuropsychopharmacology* **4**, 17–26 (1991).
11. Markou, A. & Koob, G. F. Construct validity of a self-stimulation threshold paradigm: effects of reward and performance manipulations. *Physiol. Behav.* **51**, 111–119 (1992).
12. Schulteis, G., Markou, A., Gold, L. H., Stinus, L. & Koob, G. F. Relative sensitivity to naloxone of multiple indices of opiate withdrawal: a quantitative dose–response analysis. *J. Pharmacol. Exp. Ther.* **271**, 1391–1398 (1994).
13. Schulteis, G., Markou, A., Cole, M. & Koob, G. F. Decreased brain reward produced by ethanol withdrawal. *Proc. Natl Acad. Sci. USA* **92**, 5880–5884 (1995).
14. Okamoto, M., Rosenberg, H. C. & Boisse, N. R. Withdrawal characteristics following chronic pentobarbital dosing. *Eur. J. Pharmacol.* **40**, 107–119 (1976).
15. Gellert, V. F. & Holzman, S. G. Development and maintenance of morphine tolerance and dependence in the rat by scheduled access to morphine drinking solutions. *J. Pharmacol. Exp. Ther.* **205**, 536–546 (1978).
16. Mendelson, J. H. & Mello, N. K. Medical progress: biological concomitants of alcoholism. *N. Engl. J. Med.* **301**, 912–921 (1979).
17. Malin, D. H. et al. Rodent model of nicotine abstinence syndrome. *Pharmacol. Biochem. Behav.* **43**, 779–784 (1992).
18. Hildebrand, B. E., Nomikos, G. G., Bondjers, C., Nisell, M. & Svensson, T. H. Behavioral manifestations of the nicotine abstinence syndrome in the rat: peripheral versus central mechanisms. *Psychopharmacology* **129**, 348–356 (1997).
19. Malin, D. H. et al. The nicotinic antagonist mecamylamine precipitates nicotine abstinence syndrome in the rat. *Psychopharmacology* **115**, 180–184 (1994).
20. Malin, D. H., Lake, J. R., Carter, V. A., Cunningham, J. S. & Wilson, O. B. Naloxone precipitates nicotine abstinence syndrome in the rat. *Psychopharmacology* **112**, 339–342 (1993).
21. Murrin, C. L., Ferrer, J. R., Wanyun, Z. & Haley, N. J. Nicotine administration to rats: methodological considerations. *Life Sci.* **40**, 1699–1708 (1987).
22. Benowitz, N. L. Pharmacological aspects of cigarette smoking and nicotine addiction. *N. Engl. J. Med.* **319**, 1318–1330 (1988).
23. Huston-Lyons, D. & Kornetsky, C. Effects of nicotine on the threshold for rewarding brain stimulation in rats. *Pharmacol. Biochem. Behav.* **41**, 755–759 (1992).
24. Baucó, P. & Wise, R. A. Potentiation of lateral hypothalamic and midline mesencephalic brain stimulation reinforcement by nicotine: examination of repeated treatment. *J. Pharmacol. Exp. Ther.* **271**, 294–301 (1994).
25. Nisell, M., Nomikos, G. G. & Svensson, T. H. Infusion of nicotine in the central tegmental area or the nucleus accumbens of the rat differentially affects accumbal dopamine release. *Pharmacol. Toxicol.* **75**, 348–352 (1994).
26. Pontieri, F. E., Tanda, G., Orzi, F. & Di Chiara, G. Effects of nicotine on the nucleus accumbens and similarity to those of addictive drugs. *Nature* **382**, 255–257 (1996).
27. Killen, J. D., Fortmann, S. P., Newman, B. & Varady, A. Prospective study of factors influencing the development of craving associated with smoking cessation. *Psychopharmacology* **105**, 191–196 (1991).
28. Hughes, J. R. et al. Smoking cessation among self-quitters. *Health Psychol.* **11**, 331–334 (1992).
29. Doherty, K., Kinnunen, T., Militello, F. S. & Garvey, A. J. Urges to smoke during the first month of abstinence: relationship to relapse and predictors. *Psychopharmacology* **119**, 171–178 (1995).
30. Kornetsky, C. & Esposito, R. U. Euphoric drugs: effects on the reward pathways of the brain. *Fed. Proc.* **38**, 2473–2476 (1979).

Acknowledgements. This research was funded by a Novartis grant to A.M., National Institute on Drug Abuse (NIDA, USA) grants to A.M. and G.F.K., and a HIDA Individual National Research Service Award to M.P.E.-J. This is publication number 11286-NP from The Scripps Research Institute. We thank M. Arends for assistance with manuscript preparation.

Correspondence and requests for materials should be addressed to A.M. (e-mail: amarkou@scripps.edu).

Salmonella typhi uses CFTR to enter intestinal epithelial cells

Gerald B. Pier*, Martha Grout*, Tanweer Zaidi*, Gloria Meluleni*, Simone S. Mueschenborn*, George Banting†, Rosemary Ratcliff‡, Martin J. Evans§ & William H. Colledge‡

* Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115, USA

† Department of Biochemistry and Biotechnology and Biological Sciences Research Council, Molecular Recognition Centre, University of Bristol, Bristol BS8 1TD, UK

‡ Department of Physiology, University of Cambridge, Downing Street, Cambridge CB2 3EG, UK

§ Wellcome/CRC Institute of Cancer and Developmental Biology and Department of Genetics, Tennis Court Road, University of Cambridge, Cambridge CB2 1QR, UK

Homologous mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) cause cystic fibrosis (CF). In the heterozygous state, increased resistance to infectious diseases may maintain mutant CFTR alleles at high levels in selected populations¹. Here we investigate whether typhoid fever could be one such disease. The disease is initiated when *Salmonella typhi*

enters gastrointestinal epithelial cells for submucosal translocation². We found that *S. typhi*, but not the related murine pathogen *S. typhimurium*, uses CFTR for entry into epithelial cells. Cells expressing wild-type CFTR internalized more *S. typhi* than isogenic cells expressing the most common CFTR mutation, a phenylalanine deleted at residue 508 (Δ F508). Monoclonal antibodies and synthetic peptides containing a sequence corresponding to the first predicted extracellular domain of CFTR inhibited uptake of *S. typhi*. Heterozygous Δ F508 *Cftr* mice translocated 86% fewer *S. typhi* into the gastrointestinal submucosa than wild-type *Cftr* mice; no translocation occurred in Δ F508 *Cftr* homozygous mice. The *Cftr* genotype had no effect on the translocation of *S. typhimurium*. Immunoelectron microscopy revealed that more CFTR bound to *S. typhi* in the submucosa of *Cftr* wild-type mice than in Δ F508 heterozygous mice. We conclude that diminished levels of CFTR in heterozygotes may decrease susceptibility to typhoid fever.

The entry of the *Salmonella enterica* subspecies *typhimurium* (*S. typhimurium*) into epithelial cells can be used as a model to define the bacterial and host factors involved in gastrointestinal (GI) translocation of the human-restricted pathogen *S. enterica* sub-

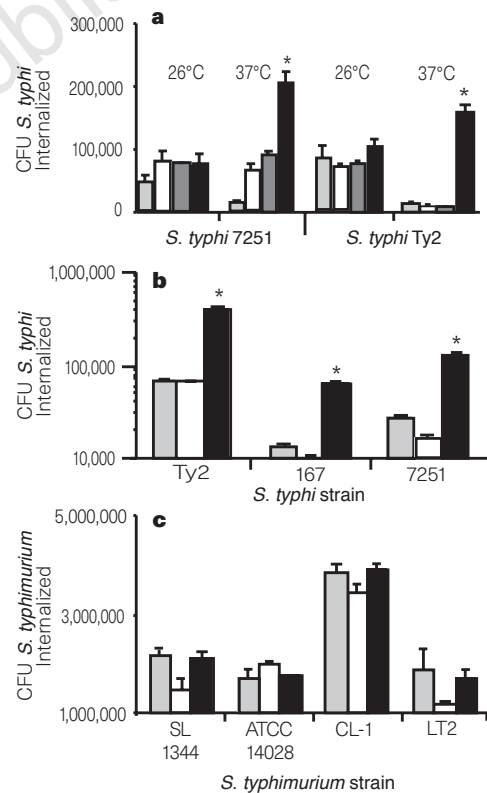


Figure 1 Uptake of different *S. typhi* and *S. typhimurium* strains by epithelial cells expressing mutant Δ F508 or wild-type *CFTR*. CFU, colony-forming units. Bars indicate means of 3–9 replicates and error bars the s.e.m. **a**, Ingestion of two *S. typhi* strains by airway epithelial cells differing in *CFTR* alleles and grown at either 26°C for 72 h or maintained at 37°C. Cells containing only Δ F508 *CFTR* alleles include: CFT1 (parental line, stippled bar), CFT1-LC3 (vector control, white bars), and CFT1-F508 (3 Δ F508 *CFTR* alleles, cross-hatched bars). The CFT1-LCFSN cells (black bars) express wild-type human *CFTR*. Asterisks indicate significantly increased uptake of *S. typhi* compared with cells with Δ F508 *CFTR* alleles only ($P < 0.001$ by ANOVA and Fisher PLSD for all pairwise comparisons). **b, c**, Uptake of indicated *S. typhi* (**b**) or *S. typhimurium* (**c**) strains by murine C127 cells transfected with either no DNA (stippled bars), or DNA encoding either human Δ F508 *CFTR* (white bars) or human wild-type *CFTR* (black bars) alleles. Asterisks indicate significantly increased uptake ($P < 0.001$, ANOVA and Fisher PLSD for pairwise comparisons). There were no significant differences ($P > 0.3$, ANOVA) for any of the *S. typhimurium* strains.

species *typhi* (*S. typhi*)². Because uptake of these two pathogens by cultured epithelial cells is comparable³, differences in this process, such as receptor usage for internalization, may be involved in the different diseases caused by these pathogens in humans. We investigated whether CFTR could be a receptor for these pathogens by using a bacterial ingestion assay at 37 °C in CFT1 epithelial cells, derived from a CF patient homozygous for the Δ F508 *CFTR* allele, and the isogenic cell line CFT1-LCFSN transfected with complementary DNA encoding wild-type *CFTR*⁴. We found that human epithelial cells expressing wild-type *CFTR* ingested significantly more *S. typhi* than cells expressing Δ F508 *CFTR* (Fig. 1a). Ingestion of *S. typhi* was the same as in wild-type cells when CFT1 cells were grown at 26 °C, a temperature that allows for apical membrane expression of the Δ F508 *CFTR* protein at ~30% of wild-type levels^{5,6} (Fig. 1a). We confirmed that the wild-type *CFTR* allele enhanced cell ingestion of *S. typhi* by measuring the uptake of three strains of *S. typhi* into C127 murine epithelioid cells expressing Δ F508 or wild-type *CFTR* alleles⁷ (Fig. 1b). In contrast, there was no difference in uptake of *S. typhimurium* by C127 cells expressing either wild-type or Δ F508 *CFTR* (Fig. 1c). Results were identical with *S. typhimurium* and the CFT1 series of epithelial cells. Thus, *S. typhi* and *S. typhimurium* enter epithelial cells by different pathways, which may be one host component influencing the pathological

differences induced by these otherwise closely related organisms.

As *S. typhi* is a GI pathogen, we evaluated *CFTR*-mediated bacterial uptake by T84 human colon carcinoma cells, which express high levels of *CFTR*. *S. typhi* ingestion by these cells is inhibited by adding *CFTR*-specific reagents to the bacterial inoculum⁸. Plasma membranes isolated from murine C127 cells containing wild-type *CFTR* inhibited internalization of *S. typhi* by T84 cells, whereas membranes from cells expressing Δ F508 *CFTR* did not (Fig. 2a). A monoclonal antibody (*CF3*)⁹ that recognizes the first extracellular domain of human *CFTR* also inhibited uptake of the bacteria, whereas antibodies against either the fourth predicted extracellular domain or a cytoplasmic domain of *CFTR* did not (Fig. 2b). Immunoblot analysis using the inhibitory *CF3* detected a protein of relative molecular mass 170,000 (M_r 170K) in immunoblot analysis of plasma membranes prepared from T84 cells and C127 cells expressing wild-type *CFTR*. In contrast, the same antibody failed to detect anything in plasma membranes from C127 cells expressing either no *CFTR* or Δ F508 *CFTR* (inset in Fig. 2b). To confirm the specificity of the *CF3* antibody, we added different synthetic *CFTR* peptides to the antibody before using it to inhibit bacterial entry into cells. Only the cognate peptide of the first extracellular *CFTR* domain restored epithelial cell uptake of *S. typhi* by inhibiting *CF3*. A 15-amino-acid peptide derived from the first

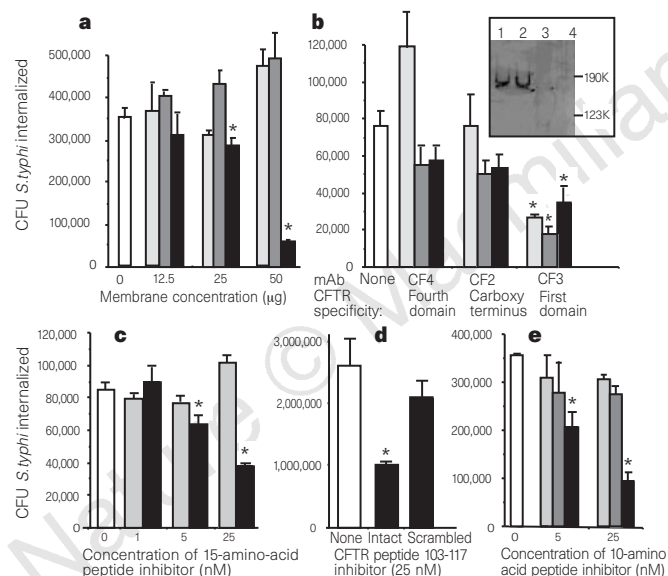


Figure 2 Inhibition of uptake of *S. typhi* Ty2 by T84 colonic epithelial cells with reagents specific to *CFTR*. **a**, Inhibition of uptake by membranes isolated from C127 murine epithelioid cells expressing no *CFTR* (stippled bars), Δ F508 *CFTR* (cross-hatched bars), or wild-type *CFTR* (black bars). **b**, Inhibition of uptake by monoclonal antibodies (mAbs) with the indicated specificity to *CFTR* domains⁹ at antibody concentrations of 60 μ g ml⁻¹ (stippled bars), 24 μ g ml⁻¹ (cross-hatched bars), or 6 μ g ml⁻¹ (black bars). Concentrations of mAb *CF3* lower than that shown did not inhibit *S. typhi* uptake. Inset, binding of mAb *CF3* to membranes extracted from (1) T84 cells; (2) C127 cells expressing wild-type human *CFTR*; (3) C127 cells expressing human Δ F508 *CFTR*; (4) untransfected C127 cells. Numbers on the right indicate relative molecular masses. **c, d**, Inhibition of uptake into T84 cell monolayers (**c**) or polarized cell cultures (**d**). In **c**, black bars represent the synthetic version of *CFTR* amino acids 103–117 and stippled bars the scrambled version of this peptide. **e**, Inhibition of uptake by a synthetic peptide corresponding to *CFTR* amino acids 108–117 (black bars), but not by a peptide of amino acids 103–112 (cross-hatched bars) or the scrambled version of peptide 103–117 (stippled bars). Bars indicate means, and error bars the s.e.m. of 6–9 replicates. Asterisks indicate differences from no inhibitor (white bar) significant at $P \leq 0.01$ (ANOVA and Fisher PLSD for pairwise comparisons).

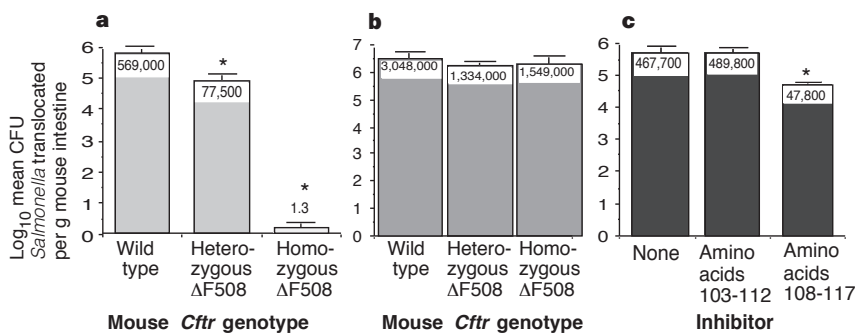


Figure 3 *S. typhi* uses *CFTR* to translocate from the GI lumen to the submucosa. Bars indicate mean of the log₁₀ CFU of *Salmonella typhi* translocated; numbers indicate the antilog of the mean; error bars indicate the s.e.m. Asterisks indicate significant differences from wild-type mice ($P < 0.001$, ANOVA and Fisher PLSD for all pairwise comparisons). **a**, Decreased translocation of *S. typhi* Ty2 from the lumen of the GI tract of mice with the indicated genotype for murine *Ctr*. **b**, No

difference in translocation of *S. typhimurium* LT-2 among wild-type, heterozygous, and homozygous Δ F508 *Ctr* mice ($P = 0.4$, ANOVA). **c**, Inhibition of translocation of *S. typhi* Ty2 from the GI lumen of BALB/c mice infected with this bacterium, plus a synthetic peptide corresponding to the indicated amino acids in the first predicted extracellular domain of *CFTR*.

CFTR extracellular domain (CFTR residues 103–117) inhibited *S. typhi* ingestion by both monolayers and polarized cultures of T84 cells (Fig. 2c, d), whereas a scrambled synthetic peptide of these residues did not. CF3, but not the other CFTR-specific antibodies, also inhibited *S. typhi* uptake by polarized T84 cell cultures ($P < 0.05$, Fisher PLSD). *S. typhi* binding to CFTR was further narrowed down to a 10-amino-acid peptide (residues 108–117) that was sufficient to inhibit ingestion (Fig. 2e). Ingestion of *S. typhi* was no better in a cell line transfected with a cDNA encoding a truncated version of wild-type CFTR (C38 cells, which lack the first 150 amino acids of CFTR) than ingestion by the parental cell line (IB3 cells) derived from a CF patient carrying the *CFTR* alleles $\Delta F508$ and W1243X (ref. 10) (data not shown). The pattern of inhibition of *S. typhi* ingestion was the same when LCFSN or HEp2 epithelial cells were used instead of T84 cells and with the same inhibitors (data not shown). Although all of these cell lines were efficient at ingesting *S. typhimurium*, we could not inhibit this uptake with CFTR-specific reagents (data not shown). These results indicate that CFTR is a major epithelial-cell receptor for internalization of *S. typhi*.

S. typhi does not cause systemic disease in mice when given orally, because *S. typhi* does not survive in murine macrophages^{11,12}; however, *S. typhi* does enter macrophages and submucosal phagocytic cells in mouse GI epithelium in much the same way as does the virulent *S. typhimurium*¹³. This property makes CFTR-mediated translocation of *S. typhi* across the murine GI epithelium a suitable model for evaluating this step in *S. typhi* pathogenesis. We have shown previously that human CFTR-specific peptides and antibodies behave comparably in bacterial ingestion assays using either human or murine CFTR-expressing target cells⁸. To investigate the effect of CFTR expression on *S. typhi* submucosal translocation, we

compared sections of murine jejunum¹⁴ from wild-type mice with littermate transgenic mice heterozygous or homozygous for the $\Delta F508$ allele of murine *Cftr*¹⁵. In $\Delta F508$ *Cftr* mice, the mutant CFTR does not leave the endoplasmic reticulum, fails to reach the plasma membrane, and is degraded¹⁶. In comparison with wild-type mice, translocation of *S. typhi* into the intestinal submucosa of $\Delta F508$ *Cftr* heterozygotes was 86% less and in homozygotes it was almost zero (Fig. 3a). There was no significant difference among wild-type, heterozygous or homozygous $\Delta F508$ *Cftr* mice in GI translocation of *S. typhimurium* (Fig. 3b), consistent with *in vitro* results¹³.

As the GI tracts of homozygous $\Delta F508$ *Cftr* mice are abnormal¹⁵, we confirmed that CFTR mediates *S. typhi* translocation by injecting these bacteria into the GI lumen of BALB/c mice together with peptides corresponding to CFTR residues that either do or do not inhibit uptake into epithelial cells (Fig. 2d, e). Inclusion of the CFTR peptide 108–117 in the infectious inoculum reduced *S. typhi* translocation by 90% compared with animals given either the bacteria alone or a control peptide of CFTR amino acids 103–112 (Fig. 3c). From the correlation of the degree of *S. typhi* translocation with *Cftr* gene dosage and the reduction in translocation that is caused by inhibiting bacterial interaction with CFTR, we conclude that CFTR is an epithelial cell receptor used by *S. typhi* to enter the GI submucosa following oral intake.

We randomly selected electron micrographs of submucosal tissue from the jejunum of wild-type and heterozygous $\Delta F508$ *Cftr* mice infected with *S. typhi* in order to quantify CFTR binding to bacteria after GI translocation. In wild-type mice, bacterial cells in the submucosa were heavily coated with CFTR after staining with monoclonal antibody CF8, which is specific for a cytosolic region of CFTR (Fig. 4a, b). Sections from heterozygous $\Delta F508$ mice showed that CFTR binding to submucosal bacteria was significantly reduced (Fig. 4c, d), indicating that the amount of CFTR reaching the plasma membrane correlates with the extent of *S. typhi* translocation from the GI lumen. These differences were quantified by counting gold particles bound per square micron of the bacterial surface (see Methods): significantly more CFTR from infected wild-type mice attached to *S. typhi* compared with CFTR from infected heterozygotes (Fig. 4f). No *S. typhi*-infected cells were seen by electron microscopy in the submucosa of homozygous $\Delta F508$ *Cftr* mice. Therefore, the smaller amount of CFTR that heterozygotes can mobilize to interact with *S. typhi* correlates with the reduced translocation of this organism across the GI epithelium. Although previous reports indicated that CF8 antibody cross reacts with unidentified antigens expressed by human cells in culture⁹, we did not find gold particles bound to GI tissues from $\Delta F508$ *Cftr* homozygotes; however, in mice with at least one wild-type *Cftr* allele, the monoclonal antibody bound to cell membranes where bacteria were not present. Under our conditions, the antibody binds to tissues only from mice expressing wild-type CFTR and is therefore specific for CFTR.

These findings indicate that *S. typhi* and *S. typhimurium* use different epithelial-cell receptors for translocation into the GI submucosa, which might explain their different host ranges and disease symptoms, and that resistance to typhoid may have selected the $\Delta F508$ allele *CFTR* to a high frequency in selected human populations. Maintenance of this allele at 4–5% in some caucasoid populations is best explained by over-dominance¹, with resistance to a particular disease conferring an advantage for the heterozygote. Heterozygotes may also be resistant to the effects of cholera toxin¹⁷: for example, $CF^{+/-}$ mice secrete less epithelial fluid following challenge with cholera toxin than do wild-type mice¹⁸. However, the acute secretory responses to a variety of agonists, including cholera toxin, are reported by others to be similar in heterozygous and wild-type CFTR mice¹⁹. Cholera has been endemic in India for centuries²⁰ but did not appear in Europe until 1832 (refs 1, 20, 21), so cholera itself is unlikely to have selected for mutant alleles of *CFTR*. Pathogens that cause diarrhoea by producing other, similar

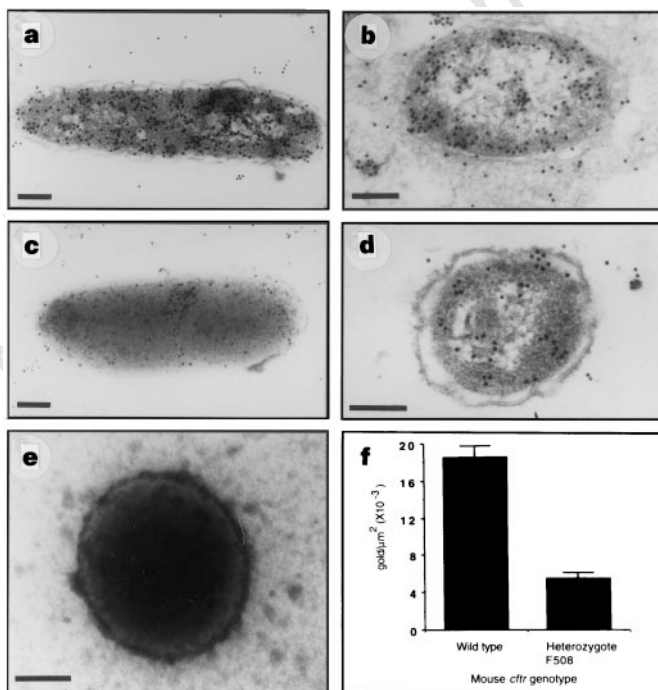


Figure 4 Immunoelectron micrographs of *S. typhi* Ty2 in the submucosal space of the GI tract of mice reacted with a monoclonal antibody to CFTR. **a, b**, *S. typhi* in the submucosa of a mouse homozygous for wild-type CFTR. **c, d**, *S. typhi* in the submucosa of a mouse heterozygous for wild-type and $\Delta F508$ CFTR. **e**, Control, with the primary antibody omitted. **f**, The number of gold particles per square micron bound to *S. typhi* Ty2 in the submucosa of mice homozygous for wild-type *Cftr* ($n = 24$ bacterial cells counted) or heterozygous for $\Delta F508$ *Cftr* ($n = 55$ bacterial cells counted). Bars indicate means and error bars s.d. the difference in gold particles bound was significant at $P < 0.001$ (*t*-test).

toxins may have selected for $\Delta F508$ CFTR. Our results show that resistance to typhoid fever could serve as the selective factor for the heterozygote advantage conferred by the $\Delta F508$ CFTR allele. □

Methods

Bacterial ingestion assays. Wild-type or $\Delta F508$ -expressing CFT1 human cell lines and C127 murine epithelioid cells were grown for 72 h at 26 °C or 24 h at 37 °C^{4,7} before bacterial-uptake assay^{6,22}. Bacterial strains were either clinical isolates or from the American Type Culture Collection. The bacterial inoculum was prepared by growing *S. typhi* overnight at 37 °C in L-broth and dilutions were made in supplemented tissue-culture medium. About $1-3 \times 10^6$ CFU in 100 μ l was added to each monolayer of 10^5 epithelial cells and $\sim 8 \times 10^6$ CFU were added to polarized cultures of T84 cells. Bacteria were allowed to invade the epithelial cells for 3–4 h at 37 °C, after which non-adherent bacteria were removed by washing. The number of ingested bacteria was quantified as described^{6,22}.

Inhibition of bacterial uptake. T84 cells (American Type Culture Collection CCL-248) were grown in a 1:1 mixture of Ham's F-12 and Dulbecco's modified Eagle's medium with 10% fetal bovine serum. They were seeded either into 96-well tissue-culture plates at 10^5 cells per well or onto Transwell polycarbonate filters (0.4- μ m pores) and grown for 6 d to form a polarized monolayer. Polarized cells were used when the electrical resistance was $\geq 2,000 \Omega \text{ cm}^{-2}$ and when $< 5\%$ of the c.p.m. of ³H-labelled mannitol added to the apical side of the cells was recovered 3 h later on the basal side²³. Plasma membranes were prepared from C127 and T84 cells as described²⁴. Membranes from C127 cells were suspended in 150 mM NaCl, 50 mM Tris (pH 7.5), 1 mM EDTA, and added at the indicated concentrations to suspensions of *S. typhi*. Monoclonal antibodies against CFTR⁹ at stock concentrations of 600 μ g ml⁻¹ were diluted from 1:10 to 1:1500 and added to bacterial inocula. Specificity of the monoclonal antibodies for CFTR in T84 and C127 cell membranes²⁴ was tested by immunoblotting⁹. Synthetic peptides mimicking the human CFTR first predicted extracellular domain were dissolved in F12 medium, and added at the indicated concentrations to bacterial inocula. These mixtures were incubated for 30 min at room temperature, then 100 μ l was added to 10^5 T84 cells growing in monolayers or to polarized T84 cells grown on filter supports. Internalized *S. typhi* were quantified as described²².

Bacterial translocation. Six-to-eight-week-old $\Delta F508$ *Cftr* transgenic mice¹⁵ were given water and a liquid elemental diet of Peptamen (Clintec Nutrition), each containing 1 mg gentamicin and 1 mg streptomycin per ml. Four days later, antibiotics were removed from the food and water and fecal pellets were checked for the presence of aerobic Gram-negative bacteria on MacConkey and *Salmonella*–*Shigella* agar plates: there was no growth on these media. Twenty-four hours later mice were killed and jejunal sections were cut into ~ 2 cm lengths, closed at each end and injected with 10^7 CFU of *S. typhi* Ty2 or *S. typhimurium* LT2. For controls, colon sections were prepared similarly. After 2 h at 37 °C in 5% CO₂, 200 μ g gentamicin in 50 μ l was injected into the lumen, and the tissue section was immersed in a solution of 400 μ g gentamicin per ml. After 1 h at 37 °C, the lumen was exposed, the tissue was weighed and then washed in 50 ml of F12 tissue-culture medium to remove gentamicin, homogenized in 0.5% Triton X-100 and then diluted for counting of bacterial cells on *Salmonella*–*Shigella* agar. Additional controls, together with the colon sections, included jejunal sections not treated with gentamicin (total growth) and jejunal sections first homogenized and then immersed in gentamicin to ensure that the antibiotic was sufficient to kill extracellular bacteria (total kill). In all cases, $> 99.9\%$ of the bacteria were killed if antibiotic was added after tissue homogenization; no *Salmonella* surviving gentamicin treatment were obtained from control colon sections. Statistical comparisons were made using the Kruskal–Wallis non-parametric ANOVA and Dunn procedure for pairwise comparison.

Inhibition by CFTR peptide of *S. typhi* translocation was tested on jejunal sections from BALB/c mice. The bacterial inoculum was delivered together with 25 nM human CFTR peptide (residues 103–112), which differs from mouse CFTR at position 112 (aspartic to glutamic acid), or another human CFTR peptide containing residues 108–117 (glutamic acid substituted by valine at position 115 in human CFTR).

Electron microscopy. For antibody-binding studies, tissues in 2% glutaraldehyde were first sectioned for electron microscopy. Nickel-coated grids with

tissue sections were placed on a 5- μ l drop of a 1:10 dilution of antibody CF8, which is specific for a 16-amino-acid region of both human and murine CFTR (which differ by a single conservative serine-to-alanine substitution at the third position of the peptide) located in the cytoplasmic domain connecting the fifth and sixth transmembrane domains and encompassing human CFTR residues 1,035–1,050 (ref. 9). Controls had no antibody. After 1 h at 25 °C, grids were washed and placed on a 5- μ l drop of rabbit antibodies raised against mouse IgM. After 30 min at room temperature, grids were washed and placed on a drop of protein A labelled with 20-nm gold particles, then incubated for 30 min. After three washes, sections were stained with osmium tetroxide and processed for electron microscopy.

Cells infected with *S. typhi* were identified on grids at a magnification (4,000–5,000 \times) too low to resolve individual gold particles, so the magnification was increased to 25,000–30,000. Two observers independently measured the bacterial surface area on the micrographs, using formulae for a circle or an ellipse, and counted the gold particles on the bacterial surface. There were no significant differences ($P = 0.8$, t -test) between the two observers' results, which were averaged to get the number of gold particles bound per μm^2 on *S. typhi* in the submucosa of wild-type *Cftr* mice or heterozygous $\Delta F508$ mice. Results were compared by t -test.

Received 30 December 1997; accepted 16 February 1998

- Bertranpetit, J. & Calafell, F. in *Variation in the Human Genome* (eds Chadwick, D. & Cardew, G.) 97–114 (Wiley, Chichester, 1996).
- Jones, B. D. & Falkow, S. Salmonellosis: host immune responses and bacterial virulence determinants. *Annu. Rev. Immunol.* **14**, 533–561 (1996).
- Mills, S. D. & Finlay, B. B. Comparison of *Salmonella typhi* and *Salmonella typhimurium* invasion, intracellular growth and localization in cultured human epithelial cells. *Microbiol. Path.* **17**, 409–423 (1994).
- Olsen, J. C. *et al.* Correction of the apical membrane chloride permeability defect in polarized cystic fibrosis airway epithelia following retroviral-mediated gene transfer. *Hum. Gene Ther.* **3**, 253–266 (1992).
- Denning, G. M. *et al.* Processing of mutant cystic fibrosis transmembrane conductance regulator is temperature-sensitive. *Nature* **358**, 761–764 (1992).
- Pier, G. B. *et al.* Role of mutant CFTR in hypersusceptibility of cystic fibrosis patients to lung infections. *Science* **271**, 64–67 (1996).
- Cheng, S. H. *et al.* Functional activation of the cystic fibrosis trafficking mutant $\Delta F508$ -CFTR by overexpression. *Am. J. Physiol.-Lung Cell. Mol. Physiol.* **12**, L615–L624 (1995).
- Pier, G. B., Grout, M. & Zaidi, T. S. Cystic fibrosis transmembrane conductance regulator is an epithelial cell receptor for clearance of *Pseudomonas aeruginosa* from the lung. *Proc. Natl Acad. Sci. USA* **94**, 12088–12093 (1997).
- Walker, J., Watson, J., Holmes, C., Edelman, A. & Banting, G. Production and characterisation of monoclonal and polyclonal antibodies to different regions of the cystic fibrosis transmembrane conductance regulator (CFTR): detection of immunologically related proteins. *J. Cell Sci.* **108**, 2433–2444 (1995).
- Flotte, T. R. *et al.* Stable *in vivo* expression of the cystic fibrosis transmembrane conductance regulator with an adeno-associated virus vector. *Proc. Natl Acad. Sci. USA* **90**, 10613–10617 (1993).
- Alpuche-Aranda, C. M., Berthiaume, E. P., Mock, B., Swanson, J. A. & Miller, S. I. Spacious phagosome formation within mouse macrophages correlates with *Salmonella* serotype pathogenicity and host susceptibility. *Infect. Immun.* **63**, 4456–4462 (1995).
- Ishibashi, Y. & Arai, T. A possible mechanism for host-specific pathogenesis of *Salmonella* serovars. *Microbiol. Path.* **21**, 435–446 (1996).
- Pascope, L. *et al.* Host restriction phenotypes of *Salmonella typhi* and *Salmonella gallinarum*. *Infect. Immun.* **63**, 4329–4335 (1995).
- Jones, B. D., Ghorri, N. & Falkow, S. *Salmonella typhimurium* initiates murine infection by penetrating and destroying the specialized epithelial M cells of the Peyer's patches. *J. Exp. Med.* **180**, 15–23 (1994).
- Colledge, W. H. *et al.* Generation and characterization of a $\Delta F508$ cystic fibrosis mouse model. *Nature Genet.* **10**, 445–452 (1995).
- Tsui, L. C. The cystic fibrosis transmembrane conductance regulator gene. *Am. J. Respir. Crit. Care Med.* **151**, S47–S53 (1995).
- Romeo, G., Devoto, M. & Galletta, L. J. V. Why is the cystic fibrosis gene so frequent? *Human Genet.* **84**, 1–5 (1989).
- Gabriel, S. E., Brigman, K. N., Koller, B. H., Boucher, R. C. & Stutts, M. J. Cystic fibrosis heterozygote resistance to cholera toxin in the cystic fibrosis mouse model. *Science* **266**, 107–109 (1994).
- Cuthbert, A. W., Halstead, J., Ratcliff, R., Colledge, W. H. & Evans, M. J. The genetic advantage hypothesis in cystic fibrosis heterozygotes: a murine study. *J. Physiol. (Lond)* **482**, 449–454 (1995).
- Pollitzer, R. *Cholera* (World Health Organization, Geneva, 1959).
- Van Heyningen, W. E. & Seal, J. R. *Cholera: The American Scientific Encounter, 1947–1980*. 1–343 (Westview, Boulder, Colorado, 1983).
- Fleiszig, S. M. J., Zaidi, T. S. & Pier, G. B. *Pseudomonas aeruginosa* invasion of and multiplication within corneal epithelial cells *in vitro*. *Infect. Immun.* **63**, 4072–4077 (1995).
- Beatty, W. L. & Sansonetti, P. J. Role of lipopolysaccharide in signaling to subepithelial polymorphonuclear leukocytes. *Infect. Immun.* **65**, 4395–4404 (1997).
- O'Riordan, C. R. *et al.* Purification and characterization of recombinant cystic fibrosis transmembrane conductance regulator from Chinese hamster ovary and insect cells. *J. Biol. Chem.* **270**, 17033–17043 (1995).
- Rosner, B. in *Fundamentals of Biostatistics* 498–503 (Duxbury, Boston, Massachusetts, 1990).

Acknowledgements. We thank J. Olsen, J. Yankaskas and L. Johnson for CFT1 cells, A. Smith and colleagues (Genzyme) for C127 cell lines, P. Zeitlin for IB3 and C38 cells, C. Lee for advice on *Salmonella* uptake assays, and C. Lee and D. Kasper for reading the manuscript. This work was supported by the NIH, the Cystic Fibrosis Foundation and the Cystic Fibrosis Trust.

Correspondence and requests for materials should be addressed by G.B.P. (e-mail: gpier@channing.harvard.edu).