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Protection against enteric salmonellosis in transgenic mice expressing a human intestinal defensin

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Genetically encoded antibiotic peptides are evolutionarily ancient and widespread effector molecules of immune defence[1–3]. Mammalian defensins, one subset of such peptides, have been implicated in the antimicrobial defence capacity of phagocytic leukocytes and various epithelial cells[4], but direct evidence of the magnitude of their *in vivo* effects have not been clearly demonstrated. Paneth cells, specialized epithelia of the small intestinal crypt, secrete abundant α-defensins and other antimicrobial polypeptides[5,6] including human defensin 5 (HD-5; also known as DEFA5)[7–9]. Although antibiotic activity of HD-5 has been demonstrated *in vitro*[9,10], functional studies of HD-5 biology have been limited by the lack of *in vivo* models. To study the *in vivo* role of HD-5, we developed a transgenic mouse model using a 2.9-kilobase HD-5 minigene containing two HD-5 exons and 1.4 kilobases of 5′-flanking sequence. Here we show that HD-5 expression in these mice is specific to Paneth cells and reflects endogenous enteric defensin gene expression. The storage and processing of transgenic HD-5 also matches that observed in humans. HD-5 transgenic mice were markedly resistant to oral challenge with virulent *Salmonella typhimurium*. These findings provide support for a critical *in vivo* role of epithelial-derived defensins in mammalian host defence.

Paneth cells reside in invaginations of the wall of the intestine called crypts of Lieberkühn, and are distributed along the length of the small intestine but are most abundant in the jejunum and ileum[6,11]. In addition to α-defensins (termed cryptdins in mice), Paneth cells synthesize and secrete other antimicrobial polypeptides, including lysosyme and secretory group II phospholipase A2 (refs 6, 11). In humans, Paneth cells express two α-defensins named HD-5 and HD-6 (refs 7, 12). The release by Paneth cells of an array of antimicrobials is thought to contribute to host defence of the small intestine by influencing the composition and controlling the numbers of microbes in the crypt and lumen[6,11]; however, *in vivo* data supporting this hypothesis are limited[13].

To investigate the *in vivo* biological role of HD-5, we have developed a transgenic model in which HD-5 is expressed in mouse small intestinal Paneth cells. A comparison of the nucleotide sequence of the two human genes expressed in Paneth cells, *HD-5* and *HD-6*, reveals a marked and rather unusual pattern of similarity—the 5′-flanking regions have sequence similarity greater than that observed in the coding regions (Fig. 1a). Reasoning that the 5′-flanking region might contain tissue-specific promoter elements, we generated HD-5 transgenic FvB mice using a 2.9-kilobase (kb) genomic fragment containing this flanking DNA and the adjacent coding exons (Fig. 1a, red bracket). Both heterozygous and homozygous HD-5 transgenic mice had normal development, fertility, intestinal histology and no observable phenotype when housed in a specific pathogen-free environment. The 1661 line contained ten copies of the transgene per diploid genome equivalent and a second line (6571) contained eight copies. These lines were indistinguishable in our analysis of transgene expression and phenotype, and for consistency, data for line 1661 are presented here. The third line (6568) contained one copy of the transgene and HD-5 expression was at levels too low for northern detection. Except for line 6568 line, which showed less HD-5 expression than would be expected, the expression of the transgene appears primarily dependent on copy number, not on site of integration.

HD-5 transgene expression was assessed by multi-tissue northern blot analysis (Fig. 1b). The pattern of HD-5 transgene expression in the small intestine reflected that of endogenous mouse and human enteric α-defensins with a greater abundance of message distally than proximally. We also compared the expression level of HD-5 messenger RNA with that of cryptdin 4 (Fig. 1c), as the latter is reported to have maximal expression in the distal small intestine[14]. We found that cryptdin 4 mRNA levels do not vary significantly between wild-type and transgenic mice, indicating that transgenic expression of HD-5 does not alter or interfere with cryptdin expression. Furthermore, the HD-5 mRNA levels in transgenic mice are comparable to expression of the endogenous cryptdin 4 in mice, and the level of transgenic HD-5 mRNA is similar to that observed in the human ileal mucosa (Fig. 1c). *In situ* hybridization analysis localized HD-5 mRNA expression to the murine crypt Paneth cells (Fig. 1d). Therefore, the HD-5 transgene, under the

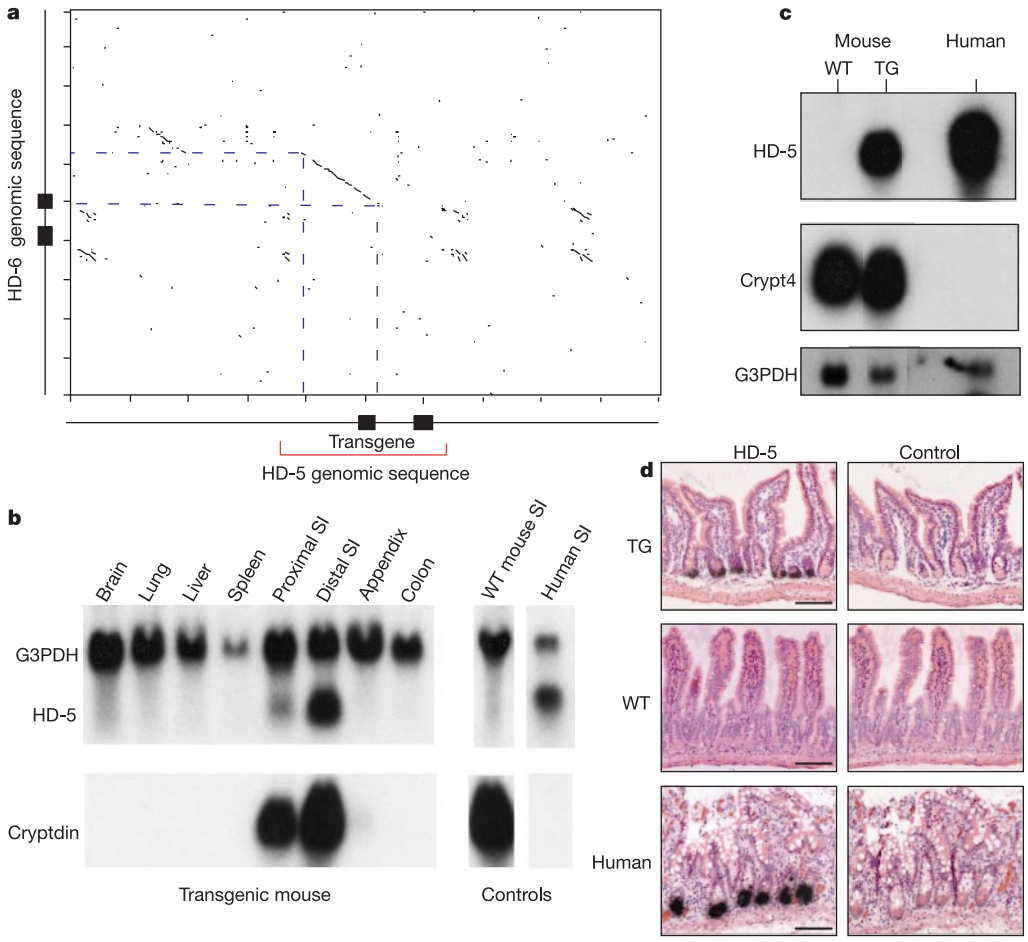

Figure 1 Germline transmission and characterization of HD-5 transgene expression in mice. **a**, Pustell analysis of HD-5 and HD-6 genomic sequences (window size 30 bp, similarity score ≥40%, hash value = 5). The flanking region immediately adjacent to exon 1 was similar in each gene (blue dashed lines). The 0.7-kb region in the *HD-6* gene in the adjacent 5′-flanking sequence was also similar to a region in the *HD-5* gene about 2 kb further upstream in its flanking sequence. A repetitive element in the intron and 3′-flanking region is repeated four times in the HD-5 genomic sequence. The transgenic genomic fragment, HG2-3e[7], used in this study is indicated by the red bracket. Exons are indicated by solid boxes. **b**, Multi-tissue northern blot analysis of transgenic HD-5 mRNA expression. A G3PDH probe[12] was used as a control for RNA quantity and integrity. Human and wild-type murine small intestinal RNA were used as controls of specific hybridization. **c**, Northern blot analysis of HD-5 transgenic and cryptdin 4 expression. RNA isolated from distal small intestine from transgenic (TG) and wild-type (WT) mice, and from human ileum were probed as described in **b**. **d**, *In situ* hybridization analysis. Expression of HD-5 mRNA by *in situ* hybridization using an antisense (AS) probe on sections of terminal ileum from a 35-day-old HD-5 transgenic mouse (TG), a wild-type age-matched control (WT) and an adult human specimen (Human) using methods as described[12]. RNase A pre-treatment controls (right panels) and sense probes (data not shown) were negative for hybridization signal. Counter stain was haematoxylin and eosin. Scale bar, 40 μm.

control of its own putative promoter, is appropriately expressed in a tissue- and cell-specific manner.

To characterize further HD-5 expression at the protein level, western blots were performed using acid-urea polyacrylamide gel electrophoresis (PAGE)[9,15,16]. The blots detected HD-5 in the distal small intestine of transgenic mice, but not in samples with equal protein loading from the proximal small intestine or in tissues from wild-type littermate controls (Fig. 2a). The predominant form of HD-5 in the distal small intestine tissue of transgenic mice migrated similarly to HD-5 isolated from human intestinal tissue and similar to recombinant proHD-5(20–94) peptide (Fig. 2a, b). The lumen of transgenic mice contained a single fast-migrating form that migrated with recombinant mature HD-5 and HD-5 isolated from human ileal lumen (Fig. 2b). Recent studies have shown that HD-5 is stored in human Paneth cells as a propeptide[9,17,18], predominantly the 20–94 form, and that during or after secretion it is proteolytically processed to a 63–94 form recovered from the intestinal lumen[9]. To characterize further both the tissue and secreted forms, HD-5 peptides were isolated from small intestinal tissue washed free of luminal contents, and from the intestinal lumen after administration of an acetylcholine agonist to elicit Paneth cell secretion[19,20]. The predominant HD-5 peptide isolated from the transgenic intestinal tissue was the 20–94 form, as determined by amino-terminal sequence and mass spectral analysis (the N-terminal sequence of the transgenic peptide was ESLQERA, identical to the human form[9]; observed $m/z = 8103.9$ daltons by matrix-assisted laser desorption/ionization (MALDI) mass spectroscopy, predicted $m/z = 8102.9$ daltons). The transgenic HD-5 peptide isolated from lumen was the 63–94 form, with N-terminal sequence (ATXYXRTG) identical to the secreted human form[9,17], and an observed mass of 3581.8 (predicted 3583.2). Thus, transgenic expression of HD-5 recapitulates expression of stored and secreted peptide as observed in humans. Using colony-forming unit (c.f.u.)-based assays, we also found that distal small-intestine-derived cationic proteins from transgenic mice have greater antibacterial activity against *Escherichia coli* compared with those from their wild-type littermates (data not shown).

We sought to determine whether the enhanced antimicrobial activity at this anatomically important site might provide protection against *S. typhimurium*, a murine enteric pathogen that is less sensitive *in vitro* to the bactericidal activity of endogenous murine cryptdins than to HD-5 (refs 9, 10, 21). HD-5 transgenic mice and age-matched FvB wild-type controls (4–5 weeks of age) were orally challenged with 1×10^8 c.f.u. virulent *S. typhimurium* (14028s). Bacteria surviving in the distal small intestine of the mice were plated on selective agar (Fig. 3a) and consistently were found to be $\geq$1 log-fold lower in the HD-5 transgenic mice compared with wild-type controls. When mice were challenged with higher numbers (1.5×10^9 c.f.u.), the phenotype conferred by transgene expression was more pronounced. At 6–24 h after inoculation the wild-type mice showed signs of progressive illness, with ruffled fur, hunched posture and diarrhoea (Fig. 3b). Mortality was 100% in wild-type control mice by 24 h after oral inoculation (Fig. 3c). In contrast, the HD-5 transgenic mice showed some initial signs of illness, but consistently recovered after 12 h with no morbidity or mortality (Fig. 3c). After this oral inoculation, numbers of recoverable *S. typhimurium* in the distal small intestine of transgenic and wild-type mice were determined (Fig. 3d). The total number of recoverable *S. typhimurium* colonies were 8×10^6 per 10 cm ileum in wild-type mice and 4×10^3 per 10 cm ileum in the HD-5 transgenic mice ($P < 0.001$, Student's *t*-test). Similarly, there were approximately 3 log-fold greater numbers of recoverable *S. typhimurium* in the faeces of wild-type control mice compared with HD-5 transgenic animals (data not shown). Thus, the *S. typhimurium* burden in the distal intestine is significantly lower in HD-5 transgenic compared with wild-type control mice, apparently as a result of HD-5 expression at this site. We suggest that HD-5 and

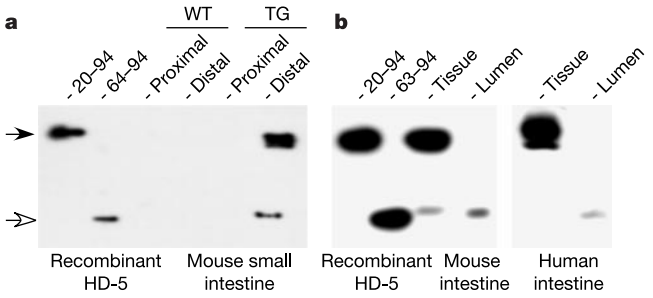

Figure 2 Western blot analysis of ileal HD-5 peptide. **a**, Western blot showing the expression pattern of HD-5 in transgenic mouse intestinal tissue. Tissue extracts from proximal and distal small intestinal tissues of wild-type and transgenic mice were loaded on 12.5% acid-urea PAGE, transferred on PVDF membranes and analysed for HD-5 immunoreactivity. Recombinant proHD-5(20–94) and mature HD-5(64–94) were used as standards (20 ng each lane). **b**, Western blot analysis of HD-5 peptide forms in small intestinal tissue and lumen of transgenic mice using recombinant HD-5 standards (left panel), and analysis of tissue and luminal forms of HD-5 in human small intestinal samples (right panel).

the aggregate of other mucosal host defence capacities of the mice combine to decrease the bacterial burden in the lumen and effectively contain the residual number of bacteria. In contrast, the significantly higher numbers of bacteria overwhelm the defence capacity in wild-type mice.

Additional studies of bacterial translocation support this conclusion. Homozygous HD-5 transgenic mice and age-matched controls were given sublethal doses of *S. typhimurium* orally. Spleens were examined for total *Salmonella* burden 3 days after inoculation (Fig. 3e). The HD-5 transgenic mice showed a tenfold reduction in *Salmonella* burden. If the effect of the HD-5 transgene expression on resistance to oral *Salmonella* were mediated in the intestinal lumen, bacterial challenge by alternative routes of administration would not show a difference when comparing transgenic with wild-type mice. We challenged groups of wild-type and transgenic mice by intraperitoneal inoculation of 10^4–10^6 c.f.u. *S. typhimurium*. Survival of transgenic and wild-type control mice was indistinguishable for each of the three levels of inocula (Fig. 3f). As data from an independent line of HD-5 transgenic mice (6571) show a highly similar phenotype, a significant effect of chromosomal insertion site of the transgene in this model system is excluded. Together, these studies are consistent with the hypothesis that the phenotype of the transgenic mice is a result of enhanced *Salmonella* killing within the intestinal lumen.

The HD-5 transgenic model has provided a means of investigating human defensin expression and function. The HD-5 transgenic mice have revealed that the promoter elements sufficient for Paneth cell expression of HD-5 are located within the 2.9-kb genomic fragment comprising the HD-5 transgene. The relative strength of the promoter elements within the transgene are probably attenuated as compared with those of the endogenous Paneth cell defensin genes, considering that the transgenic mice harbour approximately ten copies of the transgene yet yield expression levels comparable to endogenous cryptdin 4 in mouse intestine and HD-5 in human intestine. HD-5 transgenic peptide biosynthesis and processing is similar to that in humans, supporting the idea that this is a useful model to study the *in vivo* role of this human defensin. Mouse cryptdins are processed by a matrix metalloprotease called matrilysin 7 (MMP-7)[13,22], and mice rendered deficient in MMP-7 expression by targeted homologous recombination do not process cryptdins and are more susceptible to *Salmonella* challenge[13]. The processed form of HD-5 recovered from the lumen of transgenic mice, with proteolytic cleavage on the carboxyl side of arginine 62, is identical to the form recovered from the

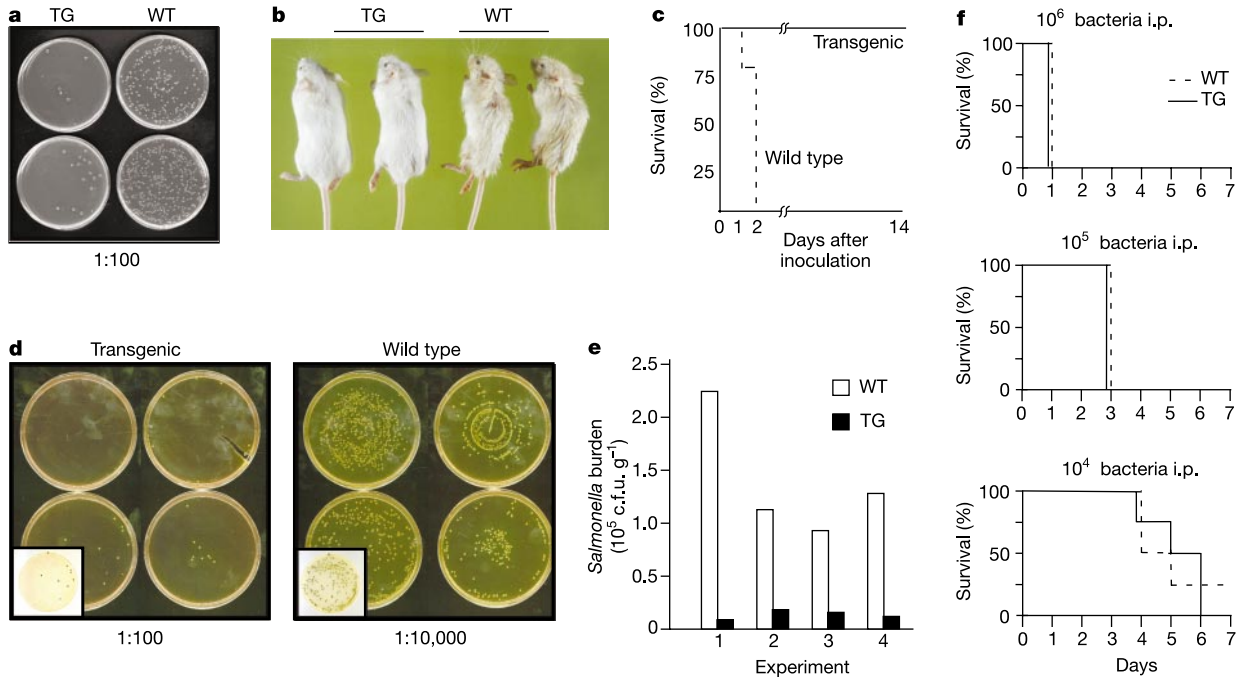

Figure 3 Challenge of HD-5 transgenic and wild-type FvB control mice with virulent *S. typhimurium*. **a**, Comparison of bacterial burden in terminal ileum in HD-5 transgenic and wild-type control mice 6 h after oral challenge with 1×10^8 c.f.u. *S. typhimurium* ($n = 6$ for each group, $P < 0.018$, Student's *t*-test). Representative plates of luminal bacteria recovered and pooled from the terminal ileum of two mice (each plate); bacteria were grown on selective medium. **b**, Comparison of HD-5 transgenic (left) and wild-type mice (right) 12 h after oral challenge with 1.5×10^9 c.f.u. *S. typhimurium*. **c**, Survival curve comparing age-matched HD-5 transgenic with wild-type control mice after oral challenge with 1.5×10^9 c.f.u. *S. typhimurium* ($n = 6$ for each group). **d**, Comparison of bacterial burden in terminal ileum in HD-5 transgenic and wild-type mice 24 h after oral challenge with 1.5×10^9 c.f.u. *S. typhimurium* (each plate represents bacteria from a single mouse). **e**, Comparison of bacterial burden in spleen of HD-5 transgenic (open) and wild-type control mice (filled), 3 days after oral challenge with 1×10^8 c.f.u. *S. typhimurium*. Four independent experiments ($n = 17$ mice for each group; pooled spleen tissue from 2–4 mice per experiment) are shown (paired *t*-test, $P = 0.026$). **f**, Survival curve comparing HD-5 transgenic (solid line) and wild-type control mice (dotted line) after intraperitoneal (i.p.) challenge with *S. typhimurium* ($n = 4$ for each group).

human intestinal lumen, where intestinal trypsin was identified as the processing enzyme[9]. Proteolytic cleavage at a canonical trypsin site is also observed for the endogenous cryptdin, cryptdin 4, but the identity of the responsible protease for the cleavage of cryptdin 4 and for the transgenic HD-5 remains to be determined. It is probable that HD-5 and cryptdin 4 are processed by the same protease, and future experiments are aimed at identifying this protein. Characterizing the processed form of transgenic HD-5 in mice deficient in MMP-7 may help to clarify this issue.

Several putative roles have been proposed for Paneth cell defensins and other antimicrobial peptides, including protection of the crypt stem cell, the regulation of the numbers and composition of the luminal microbiota, and immediate host defence against food- and water-borne enteric pathogens. In this study, we find that the expression of HD-5 in murine Paneth cells augments the endogenous innate immune capacity of mice to confer marked resistance to oral *Salmonella* infection. This is manifested by reductions in the bacterial burden in the intestinal lumen and faeces, decreased bacterial translocation, and higher survival rates after lethal *Salmonella* challenge. These results clearly support the hypothesis that Paneth cell defensins contribute significantly to mucosal host defence against infectious enteritis. Although this conclusion is more speculative, these results suggest that the natural array of antimicrobial peptides in each animal species may in part determine the pathogenicity of a particular microbe in that species. The HD-5 model may provide a means to test further this and other proposed functions of human enteric defensin peptides.

Our data offer compelling support for the emerging role of mammalian antimicrobial peptides in host defence against bacterial challenge[3]. Other reports have described complementary approaches using homologous recombination to eliminate the expression of antimicrobial peptides. Mice rendered deficient in the expression of the antimicrobial peptide cathelin-related antimicrobial peptide (CRAMP) were more susceptible to skin infections by group A *Streptococcus*[23]. Furthermore, mice deficient in the metalloprotease matrilysin, an enzyme with several functions including the processing of small intestinal defensins, are highly susceptible to oral infection by *Salmonella*[13]. However, a limitation to the loss-of-function approach for establishing *in vivo* function is that the targeted proteins often have other physiological roles that could indirectly contribute to susceptibility to infection. Here, we report a gain-of-function transgenic model, which provides data on the *in vivo* function of a human defensin peptide. Together, the complementary transgenic approaches reported previously[13] and reported here establish a key role for mucosal defensins in host defence against orally ingested pathogens. Given that antimicrobial peptides are encoded by conventional genes, future therapies may include the expression of peptides in selected cells and tissues through somatic cell gene transfer. The data reported here complement recent studies on the expression of human cathecidins and defensins through gene transfer *in vitro*, which yielded increased antimicrobial capabilities of recipient cells[24–26]. Our data highlight the effectiveness of transferring xenobiotic antimicrobial peptide genes in efforts to augment mucosal host defence. □

Methods

Generation of transgenic mice

Studies reported here were performed using protocols approved by the animal care and utilization committees at the authors' respective institutions. The DNA fragment used for

the transgene was HG2-3e, a 2.9-kb *Eco*R1 fragment of the genomic clone, containing the *HD-5* gene and flanking sequences[7]. The genomic fragment was microinjected into fertilized FvB mouse oocytes[27] and offspring were produced. DNA was isolated from portions of the tails of the FvB mouse pups and analysed by PCR using HD-5 gene-specific probes HD-5-2172s (CGGCATTTCAGAAACTGATT) and HD-5-2582a (TTCGGCAATAGCAGGTGGCT), using the following conditions in a Perkin Elmer Model 480 Thermocycler: denaturation at 94 °C for 5 min, followed by 35 cycles of 30 s at 94 °C, 30 s at 55 °C, 1 min at 72 °C, generating a 421-bp product. Three mice expressing the transgene were bred against a wild-type FvB mouse, producing independent lines of heterozygous offspring (1661, 6571, 6568) on a pure FvB genetic background. All studies reported here used mice of a pure FvB background. Homozygous mice were generated through crossbreeding of heterozygotes and were identified by genomic Southern blot analysis.

The determination of transgene copy number was made by a method described previously[28]. We made serial dilutions of the HD-5 genomic fragment. Wild-type mouse genomic DNA was used as a carrier in the dilutions of the HD-5 genomic fragment of DNA. The serial dilutions of the cloned fragment were run on a gel along with 10 μg of liver DNA from each transgenic founder line. Southern blots were hybridized to a HD-5 gene-specific probe Sig68 (GAGTGGCTCAGCCTGGGCCTGCAGGGCCACCAGGAGAATGG CAGCAA). The labelled bands were quantified using a phosphorimager. A probe to G3PDH was used to normalize for any variability in loading of DNA.

The genomic sequence encompassing the Paneth cell α-defensin genes *HD-5* (AF228730) and *HD-6* (AF314060) were compared using Pustell analysis with parameters similar to those described[12,29]. Total RNA isolated from selected mouse tissues were probed with an HD-5 probe (HSIA-309a) as described[7]. Endogenous cryptdin mRNA was detected using a probe complementary to six cryptdins (GTTTTAGTCTCTT CATCTGTGTTTTGGATAGGATCAGCCTGGACCTGG). The probe specific for cryptdin 4 mRNA was GCGGGGGCAGCAGTACAAAAATCGTATT CCACAAGTCCCACGA.

Isolation of HD-5 peptides

Small intestinal tissue from transgenic mice, fasted 12 h before they were killed, was homogenized in 20% acetic acid buffer, neutralized (pH 6) in the presence of a cocktail of protease inhibitors, diluted (1:10) and then applied to a carboxymethylcellulose (CM) resin (BioRad) using methods described previously[9,17]. To analyse and compare the tissue and luminal distribution of transgenic HD-5 peptide, 4–6-week-old mice were injected with the acetylcholine agonist aceclidine ($10\,\mu g\,g^{-1}$ in water, intraperitoneally)[30] and killed after 30 min. The small intestinal tissue was bisected lengthwise and the luminal surface was thoroughly flushed with cold normal saline; the resulting wash was diluted with ammonium acetate buffer (pH 6.0) and cationic peptides were adsorbed to CM resin. The cationic components were eluted from the CM resin in 10% acetic acid. An aliquot of the CM eluate was resolved by 12.5% acid urea gel electrophoresis, electro-blotted to a 0.2-μm polyvinylidene fluoride (PVDF) membrane, and analysed using a polyclonal HD-5 antibody and chemiluminescence detection[9]. Cationic components from the CM eluate were further fractionated using a PolyCat A weak cation exchange high-performance liquid chromatography (HPLC) column (PolyLC) as described previously[9]. HD-5 immunoreactive fractions were applied to a C-18 RP-HPLC (Vydac) column eluted with a 45-min linear gradient from 0% to 80% solvent B (0.08% trifluoroacetic acid in acetonitrile). The major HD-5 immunoreactive peak from luminal samples were fractionated by acid urea gel electrophoresis, electro-blotted to a 0.2-μm PVDF membrane, and analysed by N-terminal sequence analysis. Cationic peptides from transgenic and wild-type intestinal lumen, isolated from CM resin, were also assayed using a c.f.u. assay with *E. coli* ML35 as described[9,10].

Bacterial challenges

Bacterial inocula of *S. typhimurium* were grown in tripticase soy broth (TSB) to mid-log phase from single colonies. Bacteria were pelleted, re-suspended in fresh TSB (or PBS) and quantified using a Petroff–Hauser counting chamber. *Salmonella typhimurium* 14028s was passaged by oral inoculation in wild-type mice to enhance virulence. HD-5 homozygous FvB mice and age- and sex-matched FvB wild-type control mice were used for challenge experiments. Mice were deprived of food and bedding for 8–14 h before inoculation. Oral inoculation from a disposable syringe, or gentle gavage using a small gavage needle, was used for oral challenges ($1–5 \times 10^8$ c.f.u. of bacteria in 100 μl TSB or PBS). The mice were then returned to cages with food and bedding. If three inocula were administered, the mice were fed for 1 h after each inoculum, and after the third round of inoculum the mice were returned to cages with food and bedding. Body weight and activity were carefully monitored; moribund mice were killed and analysed by necroscopy. For the comparison of *S. typhimurium* burden in the terminal ileum, mice were killed and the terminal ileum was flushed, diluted and plated on *Salmonella–Shigella* (SS) agar (Becton Dickinson). Faecal samples were collected fresh from the animals 6–24 h after inoculation, diluted and plated on SS agar. For bacterial translocation studies, the mice were treated as above and 3 days after a single inoculation of 1×10^8 c.f.u., they were killed, their spleens isolated and homogenized, and bacteria were plated in dilution on SS agar. For intraperitoneal infection, HD-5 homozygous mice and wild-type controls ($n = 4$ per group, 6 weeks of age) were injected with 100 μl of PBS containing 10^6, 10^5 or 10^4 c.f.u. of *Salmonella*. The mice were carefully monitored and immediately killed if they became moribund.

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news and views

Protection against enteric salmonellosis in transgenic mice expressing a human intestinal defensin

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Microbiology

Gut defence

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The severity of salmonella infections depends in part on how effectively the invaders are destroyed. Incisive experiments now show that host defence in the intestine centres on the aptly named defensins.

Cells that are engaged in the antimicrobial defence of mammals and birds produce defensins, a family of structurally related antimicrobial molecules[1,2]. The production of similar small proteins is also often induced by infections in invertebrates and plants[3,4]. Because of their abundance in infected tissues and their ability to kill a variety of microbes under laboratory conditions, defensins are thought to function as natural antibiotics. Until now, however, the evidence for their contribution to antimicrobial defence in living mammals was entirely circumstantial. That changes with the appearance of the paper by Salzman *et al.* on page 522 of this issue[5]. The authors report that they performed a genetic transplant of human defensin 5 (HD-5) into mice and observed a dramatic improvement in the resistance of the mice to intestinal infection with *Salmonella typhimurium*.

Salmonella bacteria cause several types of disease in humans and animals[6]. A diarrhoeal illness contracted by eating contaminated food is the most common consequence of *S. typhimurium* infection in humans. In this disease, the bacteria are confined to the lumen and absorptive surface of the small intestine, where they cause inflammation but do not spread through the blood to other organs. However, when *S. typhimurium* infects mice, or the related bacterium *S. typhi* infects humans, they cause typhoid fever, a much more serious illness, in which the bacteria spread from the intestine to other organs. The severity of the disease depends to a large extent on the ability of the infected host to restrict the multiplication of the bacteria and to prevent them from penetrating the intestinal wall.

The intestinal tract is normally inhabited by a variety of resident bacteria growing at low density in the small intestine but abundantly in the colon. Paneth cells[7] located in crypts, tiny pits throughout the small intestine (Fig. 1), release defensins and other antimicrobial substances and contribute to the ability of fluid in the small intestine to prevent the growth of invading bacteria. Previous studies had shown that *S. typhimurium* is much more sensitive to human defensin HD-5 than to the defensins produced by mouse Paneth cells.

Salzman *et al.*[5] reasoned that if defensins function as natural antibiotics, then transgenic mice constructed to make HD-5 in their Paneth cells (HD-5 mice) should have increased resistance to infection with *S. typhimurium*. They orally infected normal and HD-5 mice with these bacteria, and observed that all HD-5 mice survived infection with doses of bacteria that killed all the normal mice within 2 days. Moreover, by 24 hours after infection, the bacterial counts in the faeces or intestines of HD-5 mice were a thousand times lower than those in normal mice, and the spread to other organs was also greatly decreased. The protective effect of HD-5 was detectable very early, with obvious differences between the HD-5 and normal mice only 6–12 hours after infection. The site of HD-5 activity was clearly in the intestine,

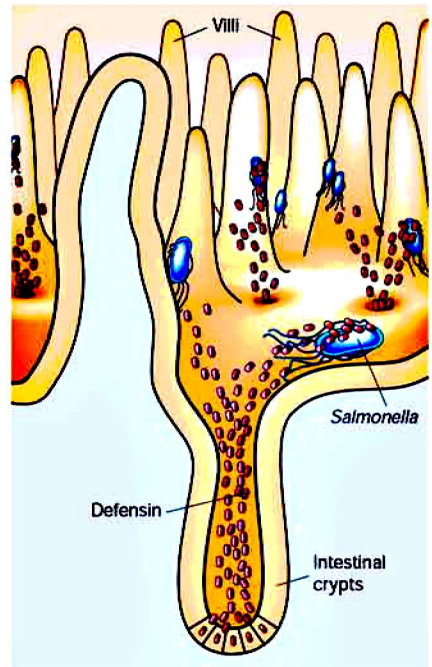

Figure 1 Bacterial invasion and the gut response. Salmonella bacteria attach to villi of the intestine but are attacked by defensins secreted from Paneth cells located in the intestinal crypts. Defensins are stored in large cellular granules, and are activated during or after release by the removal of a 'propiece' by trypsin, an enzyme released by Paneth cells. In the new work, Salzman *et al.*[5] provide solid experimental evidence that defensins act as species-specific antibiotics *in vivo*. (Redrawn from artwork by Dave Schumick, Cleveland Clinic Foundation.)

because there was no difference in the survival of HD-5 mice and normal mice when the bacteria were injected into the abdominal cavity, bypassing the intestinal secretions.

Finally, the authors also documented that the concentrations of human defensin in HD-5 mice were comparable to the concentrations of native mouse defensins, so that the observed effects were not due to unrealistically high levels of human defensins in the transgenic animals. The presence of comparable amounts of normal mouse Paneth-cell defensins, and the rapid time course of the effect of HD-5, also suggest that HD-5 acts as an antibiotic and not by indirect mechanisms such as increased recruitment of host defence cells to the infection area or priming of other immunological responses. More detailed examination of HD-5's mechanism of action in this setting should clarify this point.

Several previous studies support the idea that defensins and other antimicrobial peptides contribute to antibacterial defence in mammals. Mice that lack matrilysin, an enzyme that activates several mouse intestinal defensins, also show impaired resistance to intestinal infections[8]. However, matrilysin

deficiency is not strictly equivalent to defensin deficiency because, as well as activating defensins, matrilysin could have other functions in host defence. Mice constructed to lack another non-defensin antimicrobial peptide, CRAMP (cathelin-related antimicrobial peptide), are abnormally susceptible to skin infections with group A streptococci[9]. But CRAMP and defensins are structurally very different, so it could not be assumed that their biological roles are the same. The evidence presented by Salzman *et al.* is the strongest yet that defensins restrict microbial growth *in vivo*.

Defensins are not only made in the intestine but are abundant in other human cells and tissues. Four α-defensins closely related to HD-5 are found in human neutrophils, blood cells that specialize in the killing of bacteria and fungi in infected tissue. At least three β-defensins, which belong to a related branch of the defensin family, are secreted into the urine from the kidneys, made in infected skin, or found in other secretions that cover exposed body surfaces. Although it is likely that their functions are similar to that of HD-5, detailed studies of their specific contributions to host defence would be worthwhile. Larger human proteins, such as lysozyme and secretory phospholipase A_2, may also act as natural antibiotics in the small intestine[10] and elsewhere[11].

It has long been known that the same bacteria can cause severe, mild or no disease, depending on the mammalian species infected[12], but the reasons for these differences have remained a mystery. The study of Salzman *et al.*[5] suggests that humans infected with *S. typhimurium* develop a much milder disease than do mice because human intestinal defensins are more effective against these bacteria than are their mouse counterparts. Defensins are a rapidly evolving family of peptides, and even closely related animal species often differ in the tissues in which the defensins are made and which microbes they can kill or inhibit. *Salmonella typhimurium* (Fig. 2) is one of the most common agents of bacterial diarrhoea in humans[6], and human intestinal defensins may have evolved to be particularly effective against these bacteria. At the other extreme, humans often suffer from infections that spare other animals. By showing that a human-derived antibiotic can ameliorate an infection in mice, the new study raises the prospect that peptide antibiotics from other animal species may soon be used to help treat infections in humans. ■

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Figure 2 *Salmonella typhimurium*, shown here in culture. These bacteria usually have much more severe effects in mice than in humans.