Way, L. Wang, J.-Q. Run, A. Wang, *Genes Dev.* **5**, 2199 (1991)], except that animals were treated with 38 Gy (1 Gy = 100 rads) from an x-ray source. Transgenic strains were outcrossed to *dpy-20(e1282); him-5(e1490),* and the transgene was reisolated in a *dpy-20(e1362)* background with or without *him-5* [J. Hodgkin, H. R. Horvitz, S. Brenner, *Genetics* **91**, 67 (1979)]. Chromosomal integration of transgenic arrays stabilized but did not alter the observed phenotypes.

6. Nematodes were fixed and stained with X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) or Salmon-Gal (6-chloro-3-indolyl-β-D-galactopyranoside) (Biosynth) (*21*). Animals were examined with Nomarski optics.
7. Cell identifications were made by the size, shape, and position of cell bodies or their nuclei [J. E. Sulston and H. R. Horvitz, *Dev. Biol.* **56**, 110 (1977); J. E. Sulston, D. G. Albertson, J. N. Thomson, *ibid.* **78**, 542 (1980); J. G. White, E. Southgate, J. N. Thomson, S. Brenner, *Philos. Trans. R. Soc. London B. Biol. Sci.* **314**, 1 (1986)].
8. Transgenic *C. elegans* bearing a *goa-1*–GFP fusion, pJMOB61 (*4*), were examined, and cells were scored for green fluorescence.
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10. Movement, the stage of egg laying, and male mating efficiency were tested in +/+, *pk62/+, sy192/+, pk62, sy192,* and *pk62/sy192* animals. Movement was scored by direct observation of hermaphrodites and their tracks in a bacterial lawn. Eggs were harvested at 10-min intervals and examined with Nomarski optics. The percentage of potent males, relative to wild type, was determined by placing individual L4 males on culture dishes with six *unc-52(e444)* (*22*) L4 hermaphrodites. A male was scored as potent if cross progeny were observed after 5 days. All males were *him-5(e1490)*. *pk62* is semidominant for impotence (87% of *pk62/+* were potent, $n = 30$, Table 1), whereas *sy192* is semidominant for movement (26% of *sy192/+* were hyperactive, $n = 23$) and dominant for premature egg laying (4.0 ± 3.6 cells/egg, $n = 33$ eggs, Table 1). Hyperactivity and premature egg laying were enhanced in trans: 100% of *pk62/sy192* were hyperactive ($n = 30$) and freshly laid eggs had 1.8 ± 0.9 cells ($n = 24$). Of the *pk62/sy192* males 64% were potent, similar to *sy192* (Table 1). The plasmid pJMGo (*12*) rescues the phenotypes of *pk62* and *sy192*.
11. S. B. Masters *et al.*, *J. Biol. Chem.* **264**, 15467 (1989); M. P. Graziano and A. G. Gilman, *ibid.*, p. 15475; Y. H. Wong *et al.*, *Nature* **351**, 63 (1991). Mammalian $G\alpha_o$ containing a substitution at the equivalent glutamine (Q205L) is protected by GTP from tryptic digestion as are constitutively activated $G\alpha_s$ and $G\alpha_i$, suggesting that this substitution also results in constitutively activated $G\alpha_o$ [V. Z. Slepak, T. M. Wilkie, M. I. Simon, *J. Biol. Chem.* **268**, 1414 (1993); S. M. Strittmatter, M. C. Fishman, X.-P. Zhu, *J. Neurosci.* **14**, 2327 (1994)].
12. The plasmid pJMGo has a 9.0-kb insert in pBS+ (Stratagene) containing the *goa-1* coding sequence flanked by 5 kb of DNA at the 5′ end and 1 kb of DNA at the 3′ end (*3*). The extent of the 5′ sequence in pJMGo matches that of the *goa-1*-reporter plasmids pJMOBS.11 and pJMOB61 (*4*). For mutagenesis in vitro, we used the primer 5′-CCTTTCTGATCTAAGACCTCCCACATC-3′, corresponding to nucleotides 693 to 719 of the *goa-1* cDNA (*3*). The mutation was confirmed by limited sequencing of the resulting plasmid, pJMGoQL. To establish *syls9*, we injected pJMGoQL into *C. elegans* (*5*) at 5 μg/ml along with pMH86 (*dpy-20*) at 10 μg/ml and pBS+ at 100 μg/ml.
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14. Transgenic animals bearing multiple copies of wild-type $G\alpha_o$ (pJMGo) (*12*) were also defective in egg laying, although not as severely as *syls9* animals. We therefore introduced a frame-shift mutation into pJMGoQL upstream of the Q-L substitution: pJMGoQL was cleaved at a unique Sph site corresponding to nucleotide 176 in the *goa-1* cDNA sequence (*3*), treated with T4 DNA polymerase, and religated. Limited sequence analysis of the resulting plasmid, pJMStopnGo, showed a deletion of five base pairs at the expected position. The conceptual translation of pJMStopnGo matches the wild-type sequence through residue 27 with translation ceasing at codon 49. We injected pJMStopnGo into *C. elegans* (*5*) at 10 μg/ml along with pMH86 (*dpy-20*) at 10 μg/ml and pBS+ at 100 μg/ml. Transgenic animals bearing this control plasmid were indistinguishable from wild-type *C. elegans*, indicating that the abnormal phenotypes seen in *syls9* animals are due to expression of the activated $G\alpha_o$ protein.
15. Activated $G\alpha_o$ may also affect chemosensation: The *syls9* adults abandoned the bacterial lawn, as do mutants lacking functional chemosensory apparatus [J. Hodgkin, *Genetics* **103**, 43 (1983)]. *syls9* also resulted in abnormal pharyngeal pumping and defecation and small body size.
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20. A 4-kb Nco-Apa fragment of pJMGoQL, extending from the start site of translation to 1 kb 3′ to the *goa-1* coding region, was inserted between the Nco and Apa sites in pPD49.78 [C. C. Mello and A. Fire, in *Methods in Cell Biology:* C. elegans, D. Shakes and H. Epstein, Eds. (Academic Press, San Diego, CA, 1995, in press)] downstream of the hsp16-2 promoter [E. G. Stringham, D. K. Dixon, D. Jones, P. M. Candido, *Mol. Biol. Cell* **3**, 221 (1992)], producing pJMGoQLH. We injected pJMGoQLH at 5 μg/ml along with pMH86 (*dpy-20*) at 10 μg/ml and pBS+ at 100 μg/ml. A transgenic line bearing pJMGoQLH as an integrated array, *syls17,* was generated (*5*) and examined for its response to heat treatment, 33°C for 30 min in S basal buffer plus cholesterol (*22*).
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8 October 1994; accepted 19 January 1995

Development of a Mouse Model of *Helicobacter pylori* Infection That Mimics Human Disease

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The human pathogen *Helicobacter pylori* is associated with gastritis, peptic ulcer disease, and gastric cancer. The pathogenesis of *H. pylori* infection in vivo was studied by adapting fresh clinical isolates of bacteria to colonize the stomachs of mice. A gastric pathology resembling human disease was observed in infections with cytotoxin-producing strains but not with noncytotoxic strains. Oral immunization with purified *H. pylori* antigens protected mice from bacterial infection. This mouse model will allow the development of therapeutic agents and vaccines against *H. pylori* infection in humans.

Infection of the human stomach by *Helicobacter pylori*, a Gram-negative spiral bacterium first isolated in 1982 from a patient with chronic active gastritis (*1*), causes nearly all duodenal ulcers and most gastric ulcers and is associated with an increased risk of gastric adenocarcinoma (*2, 3*). In developing countries, *H. pylori* infection occurs early in life (often within the first 6 to 8 months) and can persist chronically; 80% of the population is infected by age 20. In developed countries, the rate of infection slowly increases with age, and 50% of people 60 years old are infected by the bacterium (*4*). However, only a minority of infected people develop signs and symptoms of gastric pathology. Host factors have been proposed to be important for the development of symptomatic gastric disease (*5*). Treatment of gastric disease is usually achieved with H2 antagonists and represents a major expense for health care systems. Recently, antibiotic therapy has been introduced to eradicate *H. pylori* infection; nevertheless, antimicrobial treatment may be limited in the future by the emergence of antibiotic-resistant strains. Vaccination thus offers the best long-term strategy for prevention and possible treatment of disease.

Clinical isolates of *H. pylori* can be divided into at least two major types (*6*). Type I bacteria express a vacuolating cytotoxin (VacA), which induces vacuole formation in epithelial cells, and an immunodominant cytotoxin-associated antigen (CagA). Type II bacteria do not express VacA or CagA. Patients with duodenal ulcers are always infected by cytotoxin-producing type I bacteria (*7, 8*); this finding suggests that only infection with type I strains is associated with the development of gastric lesions that may evolve into severe disease. These clinical observations recently were supported by the observation that lysates of type I bacteria, but not those of type II bacteria, cause gastric damage in mice and that purified VacA cy-

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totoxin causes gastric injuries when administered orally (9).

The development of new therapies and vaccines requires an understanding of the pathogenesis of *H. pylori* infection. To date, studies of pathogenesis have been limited by the absence of adequate animal models to reproduce the various aspects of *H. pylori* disease. Laboratory strains of *H. pylori* do not infect normal laboratory rodents (*10*). Thus, experimental models currently use animals such as gnotobiotic piglets (*11*), euthymic germ-free mice (*12*), athymic nude mice (*13*), or monkeys (*14*), but these animals are difficult to handle in large numbers and cannot be used to study immune responses or to develop vaccines. *Helicobacter* species other than *H. pylori*, such as *H. felis* and *H. mustelae*, have been used to infect mice (*15*) and ferrets (*16*), respectively. However, these animal models do not mimic human *H. pylori* infection and subsequent pathology because those *Helicobacter* strains do not express VacA and other virulence factors required for the induction of ulcers and inflammation (9).

We tested the ability of fresh clinical isolates and laboratory strains of *H. pylori* to infect specific pathogen-free (SPF) CD1 mice as well as conventional BALB/c and CD1 mice. Mice were inoculated orally three times at 2-day intervals with saline or different *H. pylori* strains [10^9 colony-forming units (CFUs)]. An established laboratory strain (NCTC11637) was unable to establish infection, whereas fresh clinical isolates of both type I and type II strains colonized the stomachs of the mice (Table 1). Infection was detectable in some of the mice within 1 week and in all mice within 4 to 8 weeks after the last bacterial inoculum. Bacteria isolated from mice after 2 weeks of infection were then used to infect new mice according to the same schedule. After the first week of infection with the passaged organisms, we recovered 5×10^3 to 2×10^4 CFUs per 100 mg of gastric mucus in all mice. This result demonstrates that bacteria passaged in vivo are more efficient than clinical isolates in establishing a detectable infection, possibly because selection of good colonizers occurs in vivo (Table 2). Therefore, strains that had been isolated after a 2-week passage in mice were expanded in vitro and used in subsequent studies.

During the infection study (Table 1), mice were assessed weekly for the presence of gastric damage and immune response to the colonizing strain. The histology of the gastric mucosa is shown for a representative control mouse (Fig. 1A) and for mice infected for 8 weeks with a type I strain (Fig. 1B) or a type II strain (Fig. 1C). Infection with type I strains caused gastric pathology resembling that observed in humans infected by *H. pylori* (*17*); this pathology includes loss of gastric gland architecture, erosions and ulcerations of the gastric epithelium, and infiltration of inflammatory cells within the lamina propria. These lesions appeared in the stomachs of some mice after 2 weeks and were evident in all mice after 8 weeks of infection. The histopathology observed in the mice infected with noncytotoxic type II strains consisted only of a mild

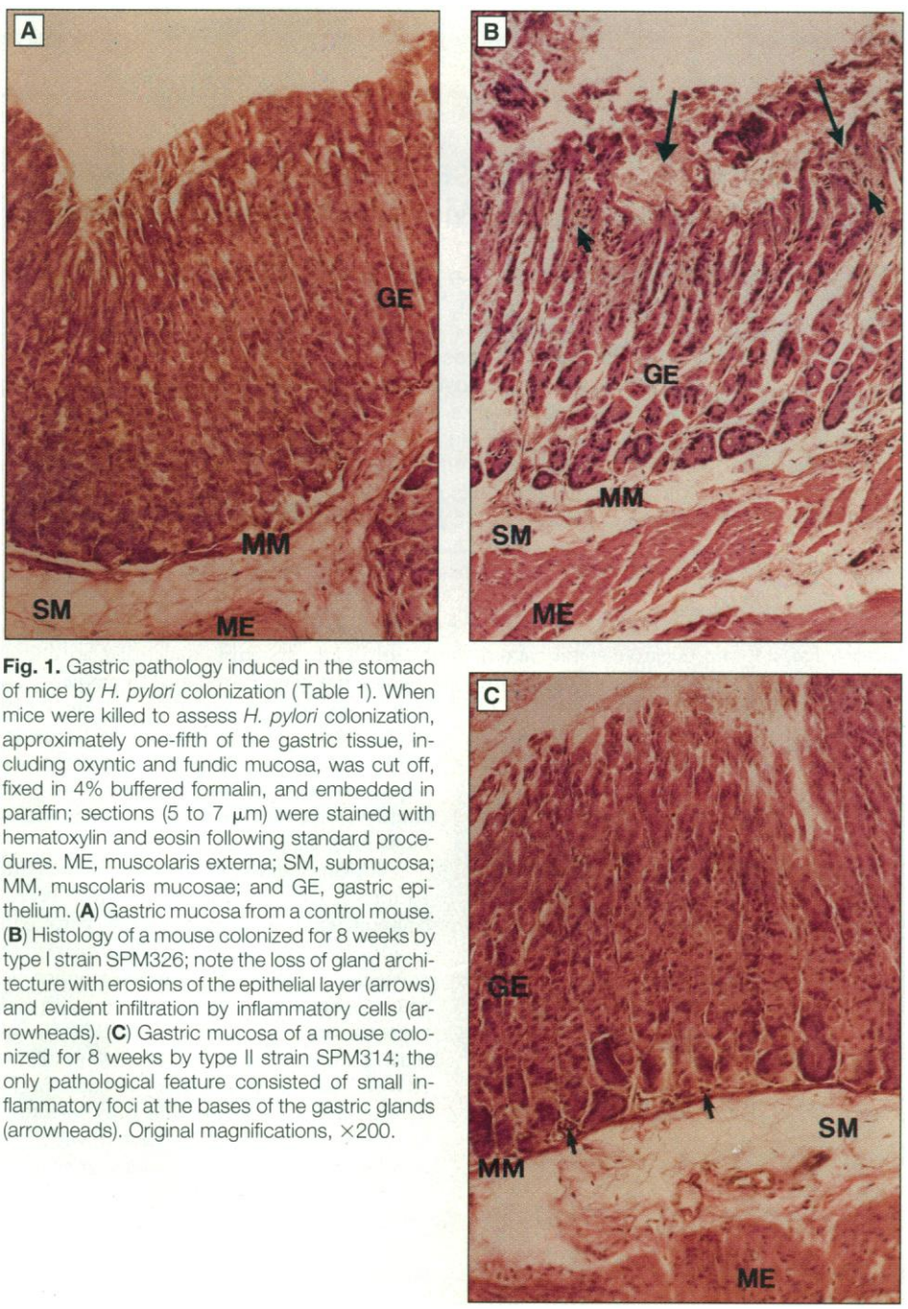

Fig. 1. Gastric pathology induced in the stomach of mice by *H. pylori* colonization (Table 1). When mice were killed to assess *H. pylori* colonization, approximately one-fifth of the gastric tissue, including oxyntic and fundic mucosa, was cut off, fixed in 4% buffered formalin, and embedded in paraffin; sections (5 to 7 μm) were stained with hematoxylin and eosin following standard procedures. ME, muscolaris externa; SM, submucosa; MM, muscolaris mucosae; and GE, gastric epithelium. (**A**) Gastric mucosa from a control mouse. (**B**) Histology of a mouse colonized for 8 weeks by type I strain SPM326; note the loss of gland architecture with erosions of the epithelial layer (arrows) and evident infiltration by inflammatory cells (arrowheads). (**C**) Gastric mucosa of a mouse colonized for 8 weeks by type II strain SPM314; the only pathological feature consisted of small inflammatory foci at the bases of the gastric glands (arrowheads). Original magnifications, ×200.

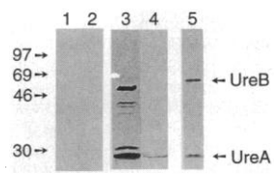

Fig. 2. Systemic immune response of a representative mouse colonized by type I strain SPM326 for 8 weeks (Table 1). Comparable results were obtained with type II strains. The immune response was assessed in the immunoblot as the total immunoglobulin response of the sera (diluted 1:150) obtained before infection (lanes 1 and 2) and 8 weeks after infection (lanes 3 and 4) against either a whole-cell preparation (10 μg) of the infecting strain (lanes 1 and 3) or against purified urease (1.5 μg) (lanes 2 and 4). Lane 5 shows the reactivity of a hyperimmune rabbit serum (diluted 1:3000) specific for the purified *H. pylori* urease (subunits UreA and UreB). Protein sizes are indicated in kilodaltons.

inflammatory infiltration. The induction of erosive lesions in the mouse gastric mucosa by infection with the type I strain is in agreement with our previous study demonstrating that lysates of type I strains, but not those of type II strains, cause acute gastric lesions when given orally to mice (9). One difference between the pathology observed in mice and that observed in humans in an early stage of infection is the lower degree of infiltration of the lamina propria of mice by polymorphonuclear leukocytes. This difference might be a result of variations in host reactivity to infection.

Infected mice showed a systemic antibody response to the colonizing strain that could be detected by an immunoblot of mice sera from the fourth week of infection. Serum reactivity against the infecting bacteria was evident in all mice after 8 weeks of infection. The whole immunoglobulin serum reaction of a representative mouse infected for 8 weeks with a type I strain is shown in Fig. 2. Serum was reacted against a total protein extract of the infecting strain (lanes 1 and 3). Preinfection serum (lane 1) did not recognize any *H. pylori* antigens, whereas the serum taken 8 weeks after infection showed reactivity against several bacterial proteins (lane 3). A strong reaction was observed against a 55-kD protein (lane 3) that probably represents a heat shock protein, homolog to Hsp60, which is also highly immunogenic in infected humans (*18*). Another major band, of about 26 kD (lane 3), comigrated with the UreA subunit of urease (lane 4), an antigen that is also immunogenic in infected humans. The observed immune response was somewhat different from that detected in humans because the 128-kD CagA antigen was not recognized (*7*). This finding may be because in this model, the immune response is studied at an early stage of infection. Indeed, it has been reported that in a human accidentally infected by *H. pylori*, the systemic immune response against CagA became detectable only 11 weeks after infection (*19*).

Because our mouse model of infection reproduces many of the features observed in humans infected with *H. pylori*, we investigated whether the model could be used for vaccine development. Therefore, mice were orally immunized with antigens that had been reported to be protective against infection with *H. felis* (*20–22*). Total bacterial lysates or purified urease were given orally to mice, with heat-labile toxin of enterotoxigenic *Escherichia coli* (LT) used as an adjuvant (*23*). Mice were then exposed to a type II strain of *H. pylori*. Complete protection was achieved in eight mice immunized with the bacterial lysate, and almost complete protection (in seven of eight mice) was obtained with purified urease (Table 3). In marked contrast, all mice that received saline, and six of the eight mice treated with adjuvant alone, were infected. The promising results obtained with the total bacterial lysate and the urease prompted us to check whether the same model could be used to develop vaccines against the bacteria that are responsible for severe disease. Therefore, antigens peculiar to type I bacteria were used to immunize mice that were then exposed to cytotoxic strains. These antigens could not be tested in the existing *H. felis* model (*20–22*) because they are not expressed by this organism (*6*). The results (Table 3) demonstrate that immunization with purified VacA protected mice from infection with a type I strain. As expected, this immunization did not protect mice from infection with type II strains (*24*). Protection from infection was also induced by urease. Histopathological analyses of protected mice showed a mild mononuclear cell infiltration (*24*) that may be a result of the immune response. The effectiveness of our vaccination model has been confirmed in 13 separate experiments involving more than 300 mice. In all, 79% of vaccinated mice were protected, whereas all control mice were infected. In mice treated with adjuvant alone, nonspecific protection was observed in 17.8% of the cases. This finding may be a result of the nonspecific immunostimulatory activity of LT (*24*).

We have described a mouse model of *H. pylori* infection that reproduces the most

Table 1. Infection of SPF CD1 mice with *H. pylori*. At intervals of 1, 2, 4, and 8 weeks after the beginning of treatment, four mice per group were killed to assess stomach colonization (*25*).

Strain	Phenotype	Number of infected mice per group			
		Week 1	Week 2	Week 4	Week 8
None		0	0	0	0
NCTC11637	Type I	0	0	0	0
SPM326	Type I	2	4	4	4
SPM342f	Type I	4	4	4	4
SPM292	Type II	1	2	4	4
SPM314	Type II	1	2	4	4

Table 2. Infection of SPF CD1 mice with *H. pylori* SPM292-2 or SPM326-2. These strains were isolated from the stomachs of mice 2 weeks after the primary colonization cycle with strains SPM292 and SPM326, respectively, and reinoculated to new mice (*25*). Groups of four mice were assessed for colonization after 1, 2, and 4 weeks. Similar results were obtained with conventional BALB/c and CD1 mice (*24*).

Strain	Phenotype	Number of infected mice per group		
		Week 1	Week 2	Week 4
SPM326-2	Type I	4	4	4
SPM292-2	Type II	4	4	4

Table 3. Protection from *H. pylori* infection by oral immunization. At days 0, 7, and 14, groups of SPF CD1 mice received an oral inoculum of 0.5 ml of saline alone or containing 10 μg of LT with or without antigens. For the mice exposed to type II bacteria, the SPM292 lysate was obtained from 10^{11} bacteria, and then an aliquot of 10^9 bacteria was administered to the mice. Urease (100 μg per treatment) was purified as described (*26*). For the mice exposed to type I bacteria, VacA (100 μg per treatment) was purified from *H. pylori* NCTC11637 as described (*9*). At days 21, 23, and 25, all the groups received an oral inoculum of 0.15 ml of sterile saline containing 10^9 CFUs of strain SPM292-2 (for mice exposed to type II bacteria) or SPM326-2 (for mice exposed to type I bacteria), and the colonization of gastric mucosa was assessed at day 35 (*27*).

Oral inoculum	Number of mice in group	Number infected	Percent protected	Number of colonies per infected mouse
	Type II (noncytotoxic) bacteria			
Saline	8	8	0	>100
LT	8	6	25	>100
SPM292 lysate + LT	8	0	100	None
Urease + LT	8	1	87.5	3
	Type I (cytotoxic) bacteria			
Saline	14	14	0	>100
LT	14	13	7.1	>100
VacA + LT	14	3	78.6	≤5
Urease + LT	13	2	84.6	≤3

relevant features of human *H. pylori* infection and pathology. Using this model, we have shown that gastric disease is induced only by infection with type I bacteria. This result is in agreement with serological data showing that most people with duodenal ulcers have antibodies specific for type I bacteria, and it confirms our previous findings with bacterial lysates or purified cytotoxin (9). Finally, we have shown that infection by both type I and type II bacteria can be prevented by oral immunization. This finding suggests that vaccines against *H. pylori* are feasible and provides the rationale to proceed with human clinical trials. The mouse model described here will allow the study of the pathogenesis of *H. pylori* infection and the development of therapeutic agents and vaccines.

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25. Mice were inoculated orally with saline or different fresh isolates of *H. pylori*. Fresh isolates of type I and type II bacteria were cultured from biopsies of patients with chronic gastritis. No correlation could be made between the type of *H. pylori* that was isolated and severity of disease, because patients were often infected by several strains. The biopsies were streaked onto Columbia agar with 5% horse blood, 0.2% cyclodextrin, and Dent's or Skirrow's antibiotic supplement (Oxoid, Basingstoke, UK), and isolation of *H. pylori* strains was performed according to standard procedures (6). Single colonies for each isolate were then grown and expanded. The primary colonization experiments were performed with strains that had never been frozen. Before infection, blood samples were drawn from all mice for serological assessment of their preimmune status. Mice were then given 0.25 ml of a solution of 0.2 M $NaHCO_3$ orally, through a sterile gastric gavage, to neutralize acidity. Through the same route, 10^9 CFUs of each strain in 0.15 ml of sterile saline were administered immediately after the bicarbonate treatment. A control group of mice received the same amount of sterile saline alone. The same treatment was repeated after 3 and 5 days. Mice were purchased from Charles River (Calco, Italy) and were housed in our animal facilities with a 12-hour light-dark schedule. Animals had free access to sterile water and food but were not allowed to eat for 24 hours before each treatment as well as before killing. The coprophagic behavior of mice was not restrained except during the fasting periods. At the time of killing, a blood sample was drawn from each mouse to assess their postinfection immune status. Animal treatments and care were given in accordance with institutional guidelines. Infection in a given mouse was assessed as follows: The stomach was removed and opened through the lesser curvature using sterile surgical instruments; the forestomach, containing nonmucosal epithelial squamous tissue, was eliminated. The whole mucous surface of the remaining gastric tissue was gently streaked onto an agar plate that was then incubated for 3 to 5 days at 37°C under microaerophilic conditions (Oxoid). Growing bacteria were identified as *H. pylori* on the basis of Gram staining and the production of urease and catalase. Some contaminant Gram-negative microaerophilic bacteria were present in the cultured plates, but they could be easily discriminated from *H. pylori* through inspection of the colony morphology, Gram staining, and the urease test. Experiments with conventional CD1 and BALB/c mice gave substantially similar results (*24*). Bacterial quantitation was done with streptomycin-resistant derivatives of the *H. pylori* strains used above, which were collected on sterile paper filters, suspended in 0.5 ml of phosphate-buffered saline, serially diluted, and then plated.
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27. All the antigens and the LT were pure as judged by SDS–polyacrylamide gel electrophoresis. The lipopolysaccharide content of the purified antigens and the LT was <0.3 endotoxin units per microgram of protein, as determined by the Limulus test (QCL-1000, BioWhittaker). Infection of mice and the determination of colonization were performed as described (*25*). The number of colonies per mouse reported in Table 3 reflects a semiquantitative measure of the number of *H. pylori* colonies present. When bacteria were confluent in most of the plate and more than 100 isolated colonies could be counted, we arbitrarily recorded the number of colonies as >100. None means absence of *H. pylori* colonies; 3 and 5 reflect the actual numbers of colonies present in the plate.

28. We thank S. Abrignani and W. Rutter for helpful discussions throughout this work; A. Covacci, D. Granoff, J. L. Telford, and D. Unutmaz for critical readings; R. Manetti for purified VacA; M. Bugnoli for purified urease; M. Pizza for LT; C. Mallia for manuscript preparation; G. Corsi for the artwork; and F. Zappalorto and A. Ruspetti for technical assistance.

17 November 1994; accepted 23 January 1995

Long-Lasting Neurotrophin-Induced Enhancement of Synaptic Transmission in the Adult Hippocampus

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The neurotrophins are signaling factors important for the differentiation and survival of distinct neuronal populations during development. To test whether the neurotrophins also function in the mature nervous system, the effects of brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and neurotrophic factor 3 (NT-3) on the strength of synaptic transmission in hippocampal slices were determined. Application of BDNF or NT-3 produced a dramatic and sustained (2 to 3 hours) enhancement of synaptic strength at the Schaffer collateral-CA1 synapses; NGF was without significant effect. The enhancement was blocked by K252a, an inhibitor of receptor tyrosine kinases. BDNF and NT-3 decreased paired-pulse facilitation, which is consistent with a possible presynaptic modification. Long-term potentiation could still be elicited in slices previously potentiated by exposure to the neurotrophic factors, which implies that these two forms of plasticity may use at least partially independent cellular mechanisms.

The neurotrophins are a group of signaling factors that are essential for the regulation of neuronal survival and differentiation during brain development. In the adult rat central nervous system, the hippocampus is a prominent site of expression of BDNF and NT-3 and their receptors (*1*). The expression of BDNF, NT-3, and their receptors can be regulated by neuronal activity (*2*–*4*), which suggests that the neurotrophins may also participate in synaptic plasticity in the adult central nervous system. Acute exposure to BDNF or NT-3, but not to NGF, rapidly potentiates the frequency of miniature synaptic events at developing neuromuscular synapses in culture (*5*), prompting us to investigate whether the neurotrophins may regulate synaptic strength in the adult brain.

We applied BDNF, NGF, and NT-3 extracellularly and examined their effects on synaptic transmission at the Schaffer collateral-CA1 neuron synapses in hippocampal slices from young adult rats (*6*). Field excitatory postsynaptic potentials (EPSPs) were

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