SUPPLEMENT MATERIAL

PCR of full length *cagA* gene

The full length *cagA* gene was PCR amplified using the following primer set: forward primer; 5′-AAA CCC GGG TAT GAC TAA CGA AAC TAT TGA CC-3′ (the *Sma*I recognition site is underlined) and reverse primer; 5′-AAA CTC GAG TTA AGA TTT TTG GAA ACC ACC-3′ (the *Xho*I site is underlined). The PCR conditions consisted of denaturation for 2 min at 95 °C, 25 amplification cycles (95 °C for 30 sec, 54 °C for 1 min, and 68 °C for 3 min), and an extension cycle of 10 min at 68 °C using Prime STAR GXL DNA polymerase (TAKARA Inc, Shiga, Japan).

Purification of East Asian-type rCagA

The harvested *E. coli* cells were suspended in a buffer containing 50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 2 mM dithiothreitol (DTT), 5 mM EDTA, 1 mM phenylmethane sulfonyl fluoride (PMSF), and 0.1 mg/mL lysozyme and then left on ice for 10 min. The *E. coli* lysate was sonicated and then centrifuged at 10,000 × *g*, 4°C, for 10 min to remove the unlysed cells. The clarified supernatant was collected and the N-terminally tagged GST-rCagA was purified by GST-tag affinity chromatography, which utilizes the binding ability of GST to glutathione sepharose 4B resin (GE Healthcare). The resin was washed with a wash solution (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1mM DTT, and 1 mM EDTA) and the rCagA was eluted following the use of PreScission protease (GE Healthcare) in 50 mM Tris-HCl [pH 7.5] that cleaves the GST-tag.

Production of an East-Asian type CagA antibody ELISA

East Asian-type rCagA (0.1 μg/well) in phosphate buffer was immobilized on Maxi-sorp 96-well plates (Thermo Fisher Scientific, Massachusetts, USA) overnight at 4 °C. The rCagA-immobilized plate was blocked with 2% (w/v) bovine serum albumin

(BSA) in phosphate buffered saline (PBS) for 1 hr at 25 °C. Human serum samples were diluted 1,000-fold in PBS with 2% (w/v) BSA and then reacted with the rCagA immobilized plate for 30 min at 25 °C. Anti-CagA rabbit IgG (1 mg/mL; Austral Líneas Aéreas, Buenos Aires, Argentina) was reacted concurrently to obtain a standard curve for calculating the amount of human IgG. The plate was washed with PBS + 0.1% (v/v) Tween-20 and then anti-human IgG conjugated horse radish peroxidase (anti-human IgG-HRP; Jackson Immuno Research Labs) and anti-rabbit IgG conjugated horse radish peroxidase (anti-rabbit IgG-HRP; Jackson Immuno Research Labs) were added to the plate. After washing the plate, ELISA peroxidase substrate 3,3',5,5'-tetramethylbenzidine (TMB; NACALAI TESQUE, Kyoto, Japan) was used for coupling and then the absorbance at 450 nm was measured. The amount of CagA antibody was calculated from the standard curve using anti-CagA rabbit IgG/anti rabbit IgG-HRP. Anti-CagA rabbit IgG was serially diluted (3.3–200 ng/mL) and reacted with rCagA-immobilized plates, and anti-rabbit IgG-HRP was then added to the plate after washing. A standard curve was prepared by plotting the concentrations of anti-CagA rabbit IgG. The amount of anti-CagA antibody was calculated from the standard curve; 1 U/mL anti-CagA antibody from human serum was comparable to 1 ng/mL anti-CagA rabbit IgG.