Supplemental information

Materials and Methods

Serum antibodies to *H. pylori* antigens. The *H. pylori* group antigen was prepared using an acid-glycine extract, as reported (24, 26). The sensitivity and specificity of these assays were 94 % and 93 % for serum IgA and IgG antibodies (26). A specific ELISA also was used to measure serum antibodies to the *H. pylori* CagA antigen, using a recombinant CagA fragment (11). An optical density ratio (ODR) of > 0.35 was considered positive, as described (25).

Specific ELISA IgG1 and IgG4 subclasses to *H. pylori* antigens was performed as previously described (27). In brief, wells were coated with the *H. pylori* pooled group antigen and incubated overnight at 25^{o}C (27). Wells then were incubated with the human serum diluted 1:200 for the IgG1 and 1:100 for the IgG4 assay. For standards, wells were coated with sheep anti-human IgG (1 mg/ml) diluted 1:100 in carbonate buffer, then incubated with concentrations ranging from 0-2000 ng/ml of purified human IgG1 or IgG4 (Southern Biotech, Birmingham AL) in PBSTT-GG. Wells were developed with peroxidase substrate solution for 30 min and optical densities determined at 405 nm. To assess immune responses to HspA, the recombinant antigen was a fusion protein containing the *E. coli* maltose binding protein (MBP) and HspA (14). The MBP-HspA fusion protein was prepared as described (13), and purified MBP alone was used for standardization. The IgG immune response to HspA was determined in parallel ELISAs using MBP-HspA or MBP alone, with subject sera diluted 1:100. The horseradish peroxidase-conjugated goat α-human IgG was diluted 1:4000 (Invitrogen Co. Carlsbad CA). Serum samples were tested in duplicate wells on each run; for each sample, the mean of the MBP optical density values was subtracted from the mean of the MBP-HspA optical density values, yielding a net optical density value (NOD). To determine the HspA-threshold value for positivity, we calculated the mean plus three standard deviations of the NOD values for 25 *H. pylori*-negative subjects from the same population. We defined HspA seropositivity as a NOD value > the calculated threshold value (0.150). This threshold value was very similar to the threshold (0.148) reported in our previous work (14), based on a group of 139 endoscoped persons known not to be colonized with *H. pylori*.

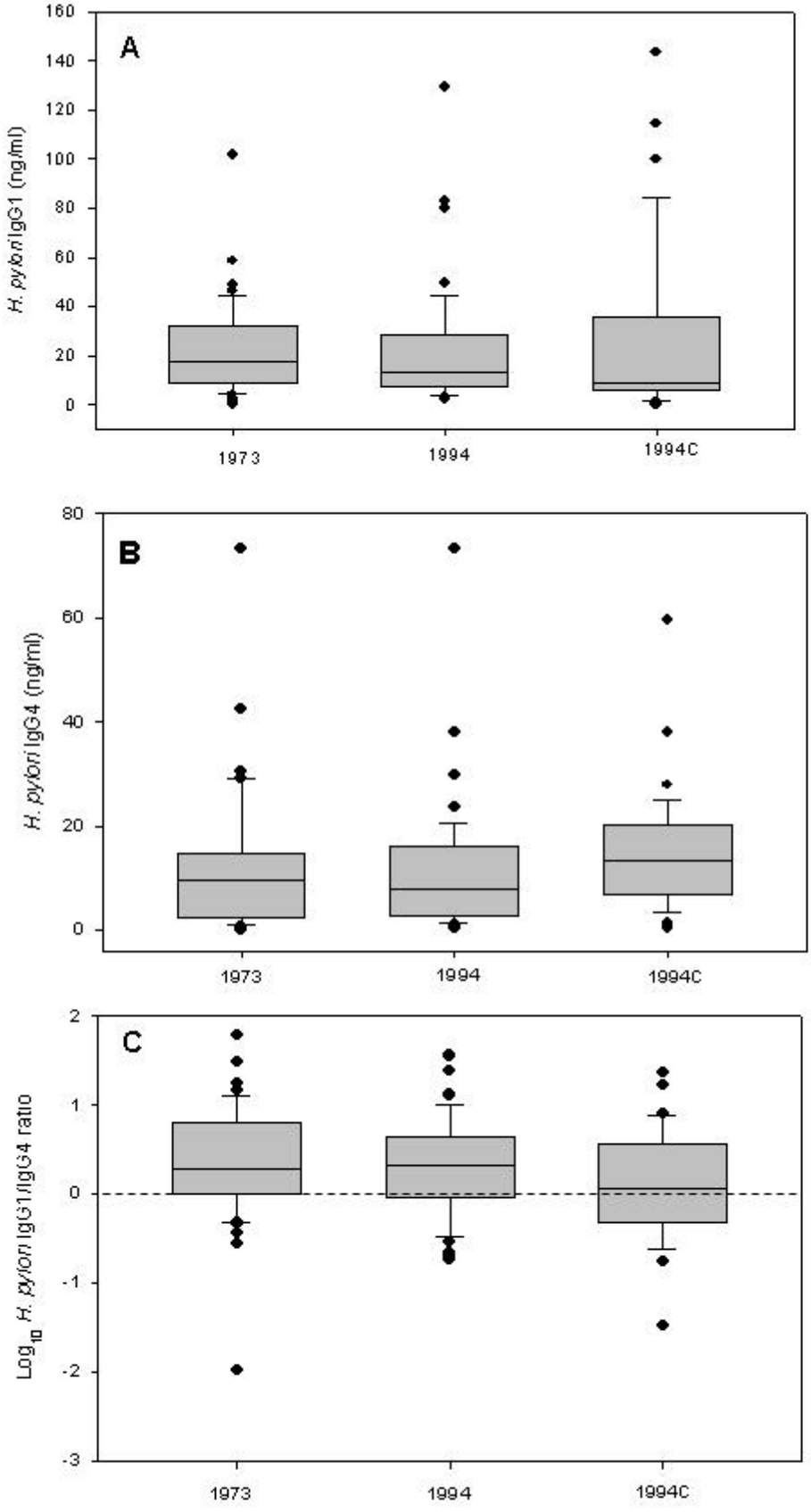
Supplemental Table 1. Relationship of the IgG subclass responses over 21 years for the paired samples, and in comparison to unpaired samples

Linear Regression Analysis[a]				
	Paired[b] samples (n=47)		Unpaired samples (n=37)	
Assessment	R	p	R	p
IgG1	0.50	<0.001	0.01	0.75
IgG4	0.55	<0.001	0.08	0.63
Ratio	0.56	<0.001	0.05	0.75

[a] Linear regression analysis of 1994 values versus the 1973 values in the same subject (paired analysis) or the 1994 values in 37 control subjects vs. 37 subjects in 1973 (unpaired analysis).

Figure Legend

Supplemental Figure 1. *H. pylori*-specific subclass responses in paired serum samples over 21 years and in a control group. IgG subclass responses specific to *H. pylori* were compared by subject group, based on the year in which the samples were obtained. Data are shown for Median+ InterQuartile Range (IQR) for IgG1 (**Panel A**), IgG4 (**Panel B**), and the $\log_{10}$ of IgG1/IgG4 ratio (**Panel C**). Circles represent values > 90^{th} or < 10^{th} percentile. The dashed line in Panel C represents the ratio when IgG1 and IgG4 are at equity.



Supplemental Figure 1