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IgA antibodies in persisting *Helicobacter pylori* infection in Finnish adults

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ABSTRACT

Most individuals infected with *Helicobacter pylori* have elevated levels of specific IgG antibodies, but only in about two-thirds of cases does the IgA titre exceed the cut-off level. The aim of this study was to determine whether *H. pylori*-infected subjects with elevated IgG levels would subsequently produce IgA antibodies, and whether elevated IgA levels increased during infection. Paired sera were available from 336 adults who took part in a large population-based health survey in 1977–1980 and a follow-up study on asthma and atopic diseases in 1997–1998 (series A). Data on paired sera from 224 adults who participated in a population-based health survey in Vammala, Finland in 1973 and who gave a follow-up blood sample in 1994 (series B) were also re-analysed. *H. pylori* IgG and IgA levels were determined with commercially available (series A) and in-house (series B) enzyme immunoassays. Twenty-one (35%) of the 60 subjects who initially had elevated levels of IgG antibodies only were found to be IgA-positive at follow-up. In those subjects whose baseline and follow-up samples were IgG- and IgA-positive, the median IgA levels increased by 48% and 22% in series A and B ($p < 0.0001$ and p 0.0241), respectively, whereas the median IgG levels did not change significantly in either series. During *H. pylori* infection, an increase in specific IgA was reflected by the increase in the number of responders and by the rise in titres.

Keywords Antibodies, *Helicobacter pylori*, IgA, IgG, serological tests, seroprevalence

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INTRODUCTION

Helicobacter pylori infection is a chronic infection that is acquired mainly in childhood [1–3]. The prevalence rates of *H. pylori* antibodies increase with age in all populations [4]. This has been explained by the persistence of childhood infections with *H. pylori*, which have decreased gradually since the early 1900s (a birth-cohort phenomenon) [2,3,5,6]. Almost all *H. pylori*-infected individuals have elevated levels of specific IgG antibodies, but only in about two-thirds of cases does the IgA titre exceed the cut-off level [3,7]. Among infected individuals, 2–7% show an elevated IgA level alone [7,8]. Production of IgA antibodies has also been associated with a CagA-positive infection [9], which is associated in turn with an increased risk of severe complications, including peptic ulcer disease [10], atrophic gastritis and intestinal metaplasia [11], and gastric cancer [12].

The prevalence rate of *H. pylori* IgA has been reported to increase with age [13,14]. The present study aimed to determine whether the increase in the number of IgA-positive individuals with age was a cohort phenomenon only, or whether *H. pylori*-infected subjects who were initially IgG-positive then proceeded to develop an IgA response. The influence of new *H. pylori*

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infections was evaluated and the level of IgA in infected subjects at baseline and after 20 years was monitored.

MATERIALS AND METHODS

Series A

The Social Insurance Institution organised a comprehensive population-based health survey in Finland (Mini-Finland Health Survey) during 1978–1980, following pilot studies carried out in 1977 [15]. A sample of 8000 individuals, representing all Finns aged ≥30 years, was studied. The sampling method comprised a two-stage stratified cluster design and the sample was self-weighting. In total, 7217 individuals (90% of the sample) participated in a basic health examination, including collection of a blood sample. Subsequently, 403 adults took part in a follow-up study concerning asthma and atopic diseases two decades later (range 17.7–20.1 years; mean 19.0 years) during 1997–1998 [16]. Of these, 139 adults either had asthma in the original survey, or had developed asthma during the follow-up period, while 264 adults served as controls. Cases and controls were matched for gender, age and area of residence. Among these 403 adults, paired serum samples were available, stored at −20°C, from 336 individuals (initial age range, 30–58 years; median age, 44 years; 207 (61.6%) females) for the present study (series A).

Series B

The present study also re-analysed data for paired sera from 224 adults who took part in a population-based health survey during 1973 in Vammala, Finland, and who gave a follow-up blood sample in 1994 (series B). Seroepidemiological findings on *H. pylori* infection and on specific IgE, based on this material, have been published previously [3,17]. Allergen-specific serum IgE antibodies against birch and timothy pollen, and cat and dog dander allergens, were determined with the radioallergosorbent test [17]. The paired sera, stored at −20°C and obtained from the 224 subjects (initial age range in 1973, 15–59 years; median age, 33 years; 131 (58.5%) females), had also been analysed for antibodies to *H. pylori*. Of the nine IgG seroconverters, eight had developed an IgA response, and of the eight IgG seroreverters, all three subjects with elevated IgA antibodies also seroreverted [3].

Serological methods

Paired sera from series A were tested for *H. pylori* IgG and IgA antibodies using Pyloriset EIA-G III and Pyloriset EIA-A III assay kits (Orion Diagnostica, Espoo, Finland). Titres ≥30 were considered positive after local validation (data not shown). With application of this threshold, the sensitivity and specificity of the Pyloriset assays were 99% and 90% for IgG, and 80% and 89% for IgA [18]. Paired serum samples were tested in parallel on the same test plates.

As reported previously [19], the cut-off titres for the in-house enzyme immunoassays used in series B were 700 for IgG and 70 for IgA. The sensitivity and specificity for the in-house enzyme immunoassays were 94% and 93% for IgG, and 73% and 95% for IgA [3].

Statistics

The statistical significance of the increase in IgA titres was examined with Wilcoxon's signed rank pair test. The increase in the number of IgA responders was evaluated with McNemar's test. Statistical analyses were performed using SPSS v. 12.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

Prevalence rates of IgA and IgG antibodies

The prevalence rates of *H. pylori* IgA and IgG increased with age in both series. The overall age-adjusted IgA prevalence was 55% during both 1977–1980 and 1997–1998 in series A, and was 30% in 1973 and 33% in 1994 in series B. For *H. pylori* IgG, the overall age-adjusted prevalence rates were 65% and 59%, respectively, in series A, and remained unchanged (45% and 46%) in series B. There was no significant difference in series B in the prevalence of *H. pylori* IgA between subjects with and without allergen-specific IgE (30% and 27%, respectively).

IgG seroconverters and seroreverters in the Mini-Finland Study

In series A, seven individuals who were originally antibody-negative had developed a positive IgG response during the subsequent two decades, and five had also become positive for IgA. The annual rate of new infections was 0.1%. The number of IgG seroreverters was 35. Among these, there was a change in IgA status from positive to negative for 15 individuals, while there was persisting IgA positivity at follow-up, despite negative IgG serology, in nine subjects.

Changes in IgA in subjects with a persisting IgG response

Within the follow-up period, there was an increase, although not statistically significant, in the number of individuals with an elevated *H. pylori* IgA response. Overall, one-third (15 of 29 in series A; six of 31 in series B) of the 60 individuals who initially had IgG only had developed an IgA response that exceeded the cut-off titre during the follow-up period (Table 1).

For the 197 individuals who showed elevated IgG and IgA antibody titres in both samples (i.e., baseline and follow-up), the median IgA titres increased by 48% ($p < 0.0001$) and 22% (p 0.0241)

Table 1. IgA antibody status during *Helicobacter pylori* infection in 268 subjects with a persisting positive IgG response

IgA status			
Initial	**Follow-up**	**Number**	**Proportion (%)**
Negative	Negative	39	14.5
Positive	Positive	197	73.5
Negative	Positive	21	7.8
Positive	Negative	11	4.1

in series A and B, respectively. In contrast, the changes in the median IgG titres (− 1% in series A; −18% in series B) were not significant (Table 2). The number of IgA seroconverters among individuals with a persisting positive IgG response (15 of 40 in series A; six of 20 in series B) was not significantly higher than that found among the IgA seroreverters (nine of 149 in series A; two of 59 in series B; p 0.1102) (Table 1).

DISCUSSION

The present study demonstrates that the increase in the prevalence rate of *H. pylori* IgA antibodies according to age [13,14] is not caused solely by the birth-cohort phenomenon and seroconverters, but is also associated with rising IgA titres during chronic infection. In several subjects, the initial normal IgA levels later rose above the cut-off level, thus increasing the proportion of IgA-seropositive subjects in older cohorts. In children, an increase in both *H. pylori* IgG and IgA titres has been observed [20]. The present findings imply that the maturation of the IgA response, indicated by rising IgA titres, still continues in adulthood, whereas the increase in IgG titres in children [20] has disappeared in adulthood. This agrees with earlier studies showing that the sensitivity of *H. pylori* IgA assays was lower than the sensitivity of tests for IgG, especially in younger age groups [18,20].

In series A, 2% of the initially antibody-negative subjects became IgG-positive during the 20-year follow-up period. This was close to the reported IgG seroconversion rate of 4% in series B, and the annual rate of new infections was quite similar [3]. The proportion of seroreverters was slightly higher in series A (10%) than in a population within series B that had not been treated actively with antimicrobial agents to cure *H. pylori* infection. In series B, only 4% of the initially antibody-positive subjects had an IgG level that had fallen below the cut-off point during the follow-up period [3].

In addition to new and cured *H. pylori* infections, the prevalence rate of *H. pylori* IgA was influenced by changes in IgA response among those who were IgG-positive throughout follow-up. Although the number of IgA seroconverters was higher than that of IgA seroreverters among subjects with a persisting positive IgG response, the difference did not reach statistical significance.

The use of frozen serum samples was based on the fact that *H. pylori* antibodies tolerate multiple cycles of freezing and thawing [21,22]. Ten cycles of freezing and thawing of known *H. pylori*-positive and -negative sera have no effect on the positivity of either IgG or IgA [21], while three thawings of sera have been reported to result in an estimated maximal reduction of 1.3% in sensitivity [22]. Thus, sera stored and thawed on several occasions can be used reliably for longitudinal research.

Although the selection (population vs. asthma patients) and age distribution of the study groups were different, and two different EIAs were used for the two series, the changes in IgA antibody titres were similar. In a separate study, which included series A, no difference was found in the prevalence of *H. pylori* IgA between subjects with and without asthma, regardless of atopy (personal unpublished observations). Furthermore, there was no significant difference in series B in the prevalence of *H. pylori* IgA antibodies between subjects with and without allergen-specific IgE. The sensitivity of the Pyloriset IgA assay is higher than that of the in-house IgA assay (80% and 73%, respectively), whereas the specificity of the Pyloriset IgA assay is lower (89% and 95%, respectively) [3,18]. However, the differences in

Table 2. Long-term changes in median IgG and IgA titres[a] during *Helicobacter pylori* infection in 197 subjects with a persisting positive IgG and IgA response

	Vammala study ***n* = 57**		**Mini-Finland study** ***n* = 140**	
	Median IgG titre	**Median IgA titre**	**Median IgG titre**	**Median IgA titre**
Initial	5500	180	2940	885
Follow-up	4500	220	2910	1310
Change of medians	− 18.2%	+ 22.2%	− 1.0%	+ 48.0%
p value for change in medians[b]	0.5000	0.0241	0.0932	< 0.0001

[a]IgG and IgA status were measured by Pyloriset EIA-GIII and -AIII (Orion Diagnostica, Espoo, Finland) in the Mini-Finland Study, and by in-house IgG and IgA enzyme immunoassays in the Vammala study.
[b]Statistical significance was examined by using Wilcoxon's signed rank pair test.

the overall age-adjusted IgA prevalences in the present study were too distinct to be explained solely by the number of false-positive samples.

The clinical importance of the IgA response is underlined when considered in association with earlier findings showing an association between *H. pylori* IgA and gastric cancer [23], and also with a CagA-positive infection [9]. As the *H. pylori* IgA response may develop later in life, many *H. pylori*-infected young individuals may have IgG only, although this does not exclude the possibility of developing an IgA response subsequently. IgA production, seen both as a greater number of responders and in higher IgA titres, has been reported to be elevated in children infected with a CagA-positive strain as compared with children infected with CagA-negative strains [24].

Individuals with a CagA-positive infection are at a higher risk of developing severe complications [10–12]. Thus, in subjects infected with a CagA-positive strain, the gastric mucosa has shown more intense inflammatory changes than in subjects infected with CagA-negative strains [24–26]. Since the seroprevalence of CagA-positive *H. pylori* infection has been found to decline more rapidly than that of CagA-negative infection in the Finnish population, especially among subjects aged <45 years [27], it will be of interest to see whether this will affect the prevalence rate and clinical importance of IgA antibodies in coming years.

In conclusion, the level of IgA increased during persisting *H. pylori* infection. This was seen both as an increased number of IgA responders and in rising antibody titres. The maturation of the IgA response in *H. pylori* infection, in contrast to the IgG response, appeared to continue into adulthood. The mechanisms behind this phenomenon remain to be studied.

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REFERENCES

1. Kuipers EJ, Pena AS, van Kamp G *et al*. Seroconversion for *Helicobacter pylori*. *Lancet* 1993; **342**: 328–331.
2. Sipponen P, Kosunen TU, Samloff IM, Heinonen OP, Siurala M. Rate of *Helicobacter pylori* acquisition among Finnish adults. *Scand J Gastroenterol* 1996; **31**: 229–232.
3. Kosunen TU, Aromaa A, Knekt P *et al*. *Helicobacter* antibodies in 1973 and 1994 in the adult population of Vammala, Finland. *Epidemiol Infect* 1997; **119**: 29–34.
4. Mégraud F. Epidemiology of *Helicobacter pylori* infection. *Gastroenterol Clin North Am* 1993; **22**: 73–88.
5. Parsonnet J, Blaser MJ, Perez-Perez GI, Hargrett-Bean N, Tauxe RV. Symptoms and risk factors of *Helicobacter pylori* infection in a cohort of epidemiologists. *Gastroenterology* 1992; **102**: 41–46.
6. Banatvala N, Mayo K, Mégraud F, Jennings R, Deeks JJ, Feldman RA. The cohort effect and *Helicobacter pylori*. *J Infect Dis* 1993; **168**: 219–221.
7. Kosunen TU, Seppälä K, Sarna S, Sipponen P. Diagnostic value of decreasing IgG, IgA, and IgM antibody titres after eradication of *Helicobacter pylori*. *Lancet* 1992; **339**: 893–895.
8. Jaskowski TD, Martins TB, Hill HR, Litwin CM. Immunoglobulin A antibodies to *Helicobacter pylori*. *J Clin Microbiol* 1997; **35**: 2999–3000.
9. Rautelin HIK, Oksanen AM, Karttunen RA *et al*. Association of CagA-positive infection with *Helicobacter pylori* antibodies of IgA class. *Ann Med* 2000; **32**: 652–656.
10. Cover TL, Glupczynski Y, Lage AP *et al*. Serologic detection of infection with *cagA*$^+$ *Helicobacter pylori* strains. *J Clin Microbiol* 1995; **33**: 1496–1500.
11. Kuipers EJ, Perez-Perez GI, Meuwissen SGM, Blaser MJ. *Helicobacter pylori* and atrophic gastritis: importance of the *cagA*-status. *J Natl Cancer Inst* 1995; **87**: 1777–1780.
12. Parsonnet J, Friedman GD, Orentreich N, Vogelman H. Risk for gastric cancer in people with CagA positive or CagA negative *Helicobacter pylori* infection. *Gut* 1997; **40**: 297–301.
13. Kosunen TU, Höök J, Rautelin HI, Myllylä G. Age-dependent increase of *Campylobacter pylori* antibodies in blood donors. *Scand J Gastroenterol* 1989; **24**: 110–114.
14. Andersen LP, Rosenstock SJ, Bonnevie O, Jorgensen T. Seroprevalence of immunoglobulin G, M, and A antibodies to *Helicobacter pylori* in an unselected Danish population. *Am J Epidemiol* 1996; **143**: 1157–1164.
15. Aromaa A, Heliövaara M, Impivaara O, Knekt P, Maatela J. *The execution of the Mini-Finland Health Survey. Part 1. Aims, methods and study population*, 1st edn. Helsinki: Social Insurance Institution, Finland, 1989; 185–199.
16. Karjalainen J, Hulkkonen J, Pessi T *et al*. The *IL1A* genotype associates with atopy in nonasthmatic adults. *J Allergy Clin Immunol* 2002; **110**: 429–434.
17. Kosunen TU, Höök-Nikanne J, Salomaa A, Sarna S, Aromaa A, Haahtela T. Increase of allergen-specific immunoglobulin E antibodies from 1973 to 1994 in a Finnish population and a possible relationship to *Helicobacter pylori* infections. *Clin Exp Allergy* 2002; **32**: 373–378.
18. Salomaa-Räsänen A, Kosunen TU, Mattila J, Sarna S, Rautelin H. Age-dependent accuracy of *Helicobacter pylori* antibody assays for adults with special emphasis on atrophic gastritis. *Clin Diagn Lab Immunol* 2004; **11**: 1185–1188.
19. Oksanen A, Veijola L, Sipponen P, Schauman K-O, Rautelin H. Evaluation of Pyloriset Screen, a rapid whole-blood diagnostic test for *Helicobacter pylori* infection. *J Clin Microbiol* 1998; **36**: 955–957.
20. Kolho KL, Korhonen J, Verkasalo M, Lindahl H, Savilahti E, Rautelin H. *Helicobacter pylori* serology at diagnosis and

follow-up of biopsy-verified infection in children. *Scand J Infect Dis* 2002; **34**: 177–182.
21. Blaser MJ, Perez-Perez GI, Lindenbaum J *et al*. Association of infection due to *Helicobacter pylori* with specific upper gastrointestinal pathology. *Rev Infect Dis* 1991; **13**(suppl 8): S704–S708.
22. Pearce DC, Peach HG, Farish SJ. *Helicobacter pylori* antibody titers in serum, plasma and successively thawed specimens: implications for epidemiological and clinical studies. *J Clin Pathol* 1996; **49**: 1017–1019.
23. Aromaa A, Kosunen TU, Knekt P *et al*. Circulating anti-*Helicobacter pylori* immunoglobulin A antibodies and low serum pepsinogen I level are associated with increased risk of gastric cancer. *Am J Epidemiol* 1996; **144**: 142–149.
24. Kolho KL, Karttunen R, Heikkilä P, Lindahl H, Rautelin H. Gastric inflammation is enhanced in children with CagA-positive *Helicobacter pylori* infection. *Pediatr Infect Dis J* 1999; **18**: 337–341.
25. Crabtree JE, Covacci A, Farmery SM *et al*. *Helicobacter pylori* induced interleukin-8 expression in gastric epithelial cells is associated with CagA-positive phenotype. *J Clin Pathol* 1995; **48**: 41–45.
26. Peek RM, Miller GG, Tham KT *et al*. Heightened inflammatory response and cytokine expression *in vivo* to *cagA*$^+$ *Helicobacter pylori* strains. *Lab Invest* 1995; **73**: 760–770.
27. Perez-Perez GI, Salomaa A, Kosunen TU *et al*. Evidence that *cagA*$^+$ *Helicobacter pylori* strains are disappearing more rapidly than *cagA*$^-$ strains. *Gut* 2002; **50**: 295–298.