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SHORT REPORT

Helicobacter pylori seropositive subjects do not show a pronounced systemic inflammatory response even in the presence of the interleukin-1 receptor antagonist gene polymorphism

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SUMMARY

The aim of this analysis was to evaluate the effects of the presence of the IL-1RA gene polymorphism and *H. pylori* infection on markers of a systemic inflammatory response taking into account virulence markers of this infection. Serum concentrations of interleukin (IL)-6, IL-8, and tumour-necrosis factor (TNF)-α of 479 occasional blood donors were not statistically significantly higher in subjects having antibodies against *H. pylori*, or more specifically against CagA and VacA, and being homozygous for the pro-inflammatory IL-1RN*2 allele compared to others after adjustment for covariates. The findings suggest that the possible pro-inflammatory effect of the IL-1RN*2 allele in combination with *H. pylori* infection is limited to the mucosal level.

Helicobacter pylori is a Gram-negative, spiral-shaped pathogenic bacterium that was first isolated and cultured from biopsy specimens by Marshall and Warren in 1983. Since 1994 infection with *H. pylori* is recognized as a major risk factor for gastric cancer by the International Agency for Research on Cancer [1]. *H. pylori* infection is probably one of the most common bacterial infections in the world. Its acquisition seems to occur in early childhood. The infection persists lifelong and in most cases causes chronic gastritis and a systemic immune response possibly leading to peptic ulceration and gastric adenocarcinoma [2].

It has been suggested that both, bacterial virulence factors (cagA pathogenicity island and vacuolating toxin VacA), as well as host polymorphisms, together with environmental and lifestyle factors determine the spectrum of clinical outcomes [3]. However, *H. pylori* causes prolonged gastric inflammation in nearly all infected persons. This inflammatory response consists of recruitment of neutrophils, T- and B-lymphocytes, plasma cells and macrophages, together with epithelial cell damage. Gastric mucosa of *H. pylori*-infected persons contains increased concentrations of pro-inflammatory cytokines [3–5], and it is supposed that CagA-positive strains of *H. pylori* induce much stronger interleukin response than CagA-negative strains [6]. Additionally, some genes which are involved in the host's inflammatory response, for instance interleukin-1 receptor antagonist (IL-1RA) gene, might be of importance in determining the strength and prolongation of this individual immune reaction. As the IL-1RA gene is polymorphic in nature, persons homozygous for the allele 2 of this gene (IL-1RN*2) develop a much more powerful and prolonged immune response compared to persons with other allele constellations [7]. It is unclear, however,

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whether this immune response might go along with a systemic inflammation, or whether it remains restricted to the gastric mucosa.

The aim of this analysis was to evaluate the combined effects of the presence of the IL-1RA gene polymorphism and *H. pylori* infection on markers of a systemic inflammatory response [serum levels of interleukin (IL)-6, IL-8, and tumour-necrosis factor (TNF)-α] taking into account virulence markers of this infection.

A total of 479 healthy adults aged 40–68 years of German nationality (occasional blood donors of the University of Ulm blood donor centre) were included in this analysis and recruited in the context of a case-control study, conducted between October 1996 and November 1997 at the University of Ulm, Germany [8]. The study was approved by the Ethics Committee of the University of Ulm and written, informed consent was obtained from all participants. Exclusion criteria were the following: history of self-reported physician-diagnosed coronary heart disease, acute state of chronic or inflammatory disease, neoplasms, acute infections, or surgery within the previous 4 weeks.

Information was obtained by means of a standardized questionnaire during a personal interview, performed by specially trained interviewers, and included details about socio-demographic aspects, medical history, current medication, self-reported weight and height, lifestyle habits (physical activity, alcohol and nicotine consumption, etc.).

Venous blood was drawn after blood donation, centrifuged, immediately aliquoted and frozen at $-70\,^{\circ}C$ until further analysis. Specific *H. pylori* immunoglobulin G (IgG) was determined using a commercial ELISA (*H. pylori*-IgG-ELISA, Medac Co., Wedel, Germany). Among IgG-positive subjects, humoral response to CagA and VacA proteins was assessed by Western blot (*H. pylori* Western Blot, AID GmbH, Strassberg, Germany) according to the manufacturer's instructions. In addition, markers of a systemic inflammatory response (IL-6, IL-8, TNF-α) were measured by ELISA (Quantikine, R&D Systems, Wiesbaden, Germany). IL-1RA gene polymorphisms were determined by PCR as described elsewhere [9]. All laboratory analyses were performed in blinded fashion.

A linear regression method was employed to estimate the association of the seroprevalence of antibodies against *H. pylori*, its virulence factors (CagA, VacA) and IL-1RA gene polymorphism with serum concentrations of IL-6, IL-8, and TNF-α after adjustment for age and gender. Subjects homozygous for allele 2 of the IL-1RA gene (IL-1RN*2) were grouped against all other allele constellations in statistical analysis.

Table 1. *Main characteristics of the study population*

	n	%
n	479	
Age (years), mean (S.D.)	55·8 (7·2)	
Males	359	74·9
Females	120	25·0
Family status married	402	83·9
School education		
<9 years	281	59·3
10–12 years	150	31·7
>13 years	43	9·0
Alcohol consumption		
Never	41	8·6
Occasionally	299	62·8
Daily	136	28·5
Smoking status		
Never smoker	210	43·8
Ex-smoker	201	41·9
Current smoker	68	14·2
BMI (kg/m²), mean (S.D.)	26·3 (3·2)	
Underweight (<20)	7	1·5
Normal weight (20–25)	180	37·6
Overweight (25–30)	238	49·7
Obesity (>30)	54	11·3
H. pylori status (IgG)		
Negative	329	68·7
Positive	150	31·3
CagA status		
Negative	390	81·4
Positive	89	18·6
VacA status		
Negative	394	82·3
Positive	85	17·7
IL-1RN*2 allele	30	6·3

Table 1 shows the main characteristics of the study population. The mean age of participants, of whom 25% were female, was 55·8 years. A total of 31·3% of subjects had a positive *H. pylori* IgG titre, 18·6% were CagA positive and 17·8% were VacA positive. The prevalence of homozygosity for the pro-inflammatory IL-1RN*2 allele was 6·3%, which is in line with other studies [7]. *H. pylori* seroprevalence in subjects with IL-1RN*2 and IL-other genotypes was 17% and 32% respectively ($P=0{\cdot}07$), showing a tendency to lower prevalence in subjects with a IL-1RA polymorphism.

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Table 2. *Individual and joint impact of the IL-1RA gene polymorphism and* H. pylori (*HP*) *status and virulence factors on markers of a systemic inflammatory response*

Factor	*n*	Adjusted β-coefficient (S.E.)†		
		IL-6	IL-8	TNF-α
IL-1RA and *HP* serostatus				
IL-other‡ and *HP* negative	304	Reference	Reference	Reference
IL-other‡ and *HP* positive	145	−0·07 (0·07)	0·07 (0·07)	0·03 (0·06)
IL-1RN*2 and *HP* negative	25	−0·06 (0·15)	0·05 (0·15)	−0·03 (0·11)
IL-1RN*2 and *HP* positive	5	0·08 (0·32)	−0·41 (0·31)	0·10 (0·25)
IL-1RA, *HP* and CagA serostatus				
IL-other‡ and *HP* negative	304	Reference	Reference	Reference
IL-other‡ *HP* positive and CagA negative	61	−0·15 (0·10)	0·01 (0·10)	0·05 (0·08)
IL-other‡ *HP* positive and CagA positive	81	−0·02 (0·09)	0·11 (0·08)	0·02 (0·07)
IL-1RN*2 and *HP* negative	25	−0·06 (0·15)	0·05 (0·15)	−0·04 (0·11)
IL-1RN*2 *HP* positive and CagA negative	2	0·16 (0·41)	−0·50 (0·40)	0·25 (0·32)
IL-1RN*2 *HP* positive and CagA positive	3	−0·04 (0·50)	−0·27 (0·49)	−0·12 (0·39)
IL-1RA, *HP* and VacA serostatus				
IL-other‡ and *HP* negative	304	Reference	Reference	Reference
IL-other‡ *HP* positive and VacA negative	57	−0·13 (0·10)	0·03 (0·10)	0·04 (0·08)
IL-other‡ *HP* positive and VacA positive	88	−0·03 (0·09)	0·10 (0·09)	0·02 (0·07)
IL-1RN*2 and *HP* negative	25	−0·06 (0·15)	0·05 (0·15)	−0·03 (0·11)
IL-1RN*2 *HP* positive and VacA negative	3	0·20 (0·50)	−0·34 (0·49)	0·14 (0·39)
IL-1RN*2 *HP* positive and VacA positive	2	−0·01 (0·41)	−0·45 (0·40)	0·07 (0·32)

† Adjusted for age and gender (none of the β-coefficients was statistically significantly different from the reference group, $P < 0·05$).

‡ All other alleles summarized except homozygous for allele 2 of the IL-1RA gene (IL-1RN*2).

In Table 2 the results of regression analysis are shown. Serum concentrations of IL-6, IL-8, and TNF-α were not statistically significantly different; first, in subjects being seropositive and having the IL-other genotype ($n = 145$) compared to seronegative subjects ($n = 304$), and second, in subjects having antibodies against *H. pylori*, or more specifically against CagA and VacA, and being homozygous for the pro-inflammatory IL-1RN*2 allele ($n = 5$) compared to others after adjustment for age and gender. Further adjustment for other covariates (school education, alcohol consumption, smoking status and body mass index) did not alter the results.

In conclusion, our results do not support the hypothesis that chronic infection with a virulent *H. pylori* strain, alone or in combination with homozygosity for the pro-inflammatory IL-1RN*2 allele leads to a stronger systemic inflammatory response in healthy subjects and are consistent with previous indications that the pro-inflammatory effect of *H. pylori* infection does not induce systemic inflammatory response, but may be restricted to the mucosal level only [10]. Our results corroborate these findings and, furthermore, indicate the absence of a systemic inflammatory response even in the presence of homozygosity for the pro-inflammatory IL-1RN*2 allele.

REFERENCES

1. International Agency for Research on Cancer. Infection with *Helicobacter pylori*. In: Schistosomes, liver flukes and *Helicobacter pylori*. Lyon: IARC Monogr Eval Carcinog Risks Hum 1994; **60**: 177–240.
2. Rothenbacher D, Brenner H. Burden of *Helicobacter pylori* and *H. pylori* related diseases in developed countries: recent developments and future implications. Microbes Infect 2003; **5**: 693–703.
3. Suerbaum S, Michetti P. *Helicobacter pylori* infection. N Engl J Med 2002; **347**: 1175–1186.
4. Crabtree JE, Wyatt JI, Trejdosiewicz LK, et al. Interleukin-8 expression in *Helicobacter pylori* infected, normal, and neoplastic gastroduodenal mucosa. J Clin Pathol 1994; **47**: 61–66.
5. Crabtree JE, Shallcross TM, Heatley RV, Wyatt JI. Mucosal tumor necrosis factor alpha and interleukin-6

https://doi.org/10.1017/S095026880400319X Published online by Cambridge University Press

in patients with *Helicobacter pylori* associated gastritis. Gut 1991; **32**: 1473–1477.
6. Yamaoka Y, Kita M, Kodama T, Sawai N, Kashima K, Imanishi J. Induction of various cytokines and development of severe mucosal inflammation by cagA gene positive *Helicobacter pylori* strains. Gut 1997; **41**: 442–451.
7. Witkin SS, Gerber S, Ledger WJ. Influence of interleukin-1 receptor antagonist gene polymorphism on disease. Clin Infect Dis 2002; **34**: 204–209.
8. Koenig W, Rothenbacher D, Hoffmeister A, et al. Infections with *Helicobacter pylori* is not a major independent risk factor for stable coronary heart disease: lack of a role of CagA-positive strains and absence of a systemic inflammatory response. Circulation 1999; **100**: 2326–2331.
9. Francis SE, Camp NJ, Dewberry RM, et al. Interleukin-1 receptor antagonist gene polymorphism and coronary artery disease. Circulation 1999; **99**: 861–866.
10. Brenner H, Berg G, Frohlich M, Boeing H, Koenig W. Chronic infections with *Helicobacter pylori* does not provoke major systemic inflammation in healthy adults: results from a large population-based study. Atherosclerosis 1999; **147**: 399–403.