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Significant increase in antigastric autoantibodies in a long-term follow-up study of *H. pylori* gastritis

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Abstract In 30% of *H. pylori*-infected patients a certain type of antigastric autoantibodies, reacting against canalicular structures within human parietal cells, is detectable. Furthermore, it has been shown that these autoantibodies are correlated with atrophy of the mucosa in the corpus. The aim of this study was to analyse the prevalence of these anticanalicular autoantibodies (ACAB) and their significance for development of gastric mucosa atrophy in a 12-year follow-up period. Gastric biopsy specimens from 62 persons in Saaremaa Island, Estonia, were collected in 1997 and assessed independently by two pathologists in accordance with the updated Sydney system. The sera of these persons were immunohistochemically screened for ACAB and for classic parietal cell antibodies (PCA). In addition, for 37 of the 62 persons, gastric biopsies and sera collected 12 years earlier (1985) were investigated in an analogous manner. ACAB increased significantly, from 8 out of 37 in 1985 to 17 out of 37 in 1997 (P=0.004; McNemar test). In 1997 a significant correlation existed between the presence of ACAB and corpus mucosa atrophy (19 out of 30 versus 10 out of 32 without atrophy; P=0.01; odds ratio (OR)=3.8, 95% CI 1.4–10.6). However, no correlation was found between ACAB and development of atrophy in the period from 1985 to 1997. All 37 persons were PCA negative in 1985, whereas in 1997, 2 turned out to be PCA positive. ACAB increased significantly with duration of *H. pylori* gastritis. The correlation between ACAB and presence of gastric corpus atrophy was confirmed. However, it is possible that ACAB are the consequence of and not a causative factor in gastric mucosa atrophy, insofar as the association of ACAB with progression of corpus atrophy was not significant.

Key words Anticanalicular autoantibodies · Corpus atrophy · Follow-up of gastritis · *Helicobacter pylori* · Parietal cell antibodies

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Introduction

H. pylori gastritis leads to the development of gastric mucosa atrophy [11, 17, 18, 24, 26]. Furthermore, recent studies have shown that a considerable proportion of *H. pylori*-infected patients develop autoimmune reactions against gastric epithelial cells [4, 14, 15, 23]. In particular, anticanalicular autoantibodies (ACAB) reacting against canalicular structures within human parietal cells can be demonstrated in 30% of infected patients. Gastric H^+,K^+-adenosine triphosphatase (H^+,K^+-ATPase) represents a major autoantigen of these autoantibodies. This particular type of antigastric autoimmunity is associated with atrophic corpus gastritis [2, 4, 5, 15].

It has been speculated that molecular mimicry between *H. pylori* and the host plays some part in the formation of ACAB in *H. pylori* gastritis [1, 14, 15, 23]. However, more recent data have provided evidence that pathogenic pathways other than molecular mimicry may also be responsible for this type of antigastric autoimmunity [2, 6].

The aim of the present study was to analyse the prevalence of ACAB and their significance for development of gastric corpus mucosa atrophy in a 12-year follow-up in an adult population from Saaremaa Island in Estonia. The study is an extension of earlier long-term follow-up research into chronic gastritis [12, 25–27] and parietal cell antibodies (PCA) in different Estonian populations

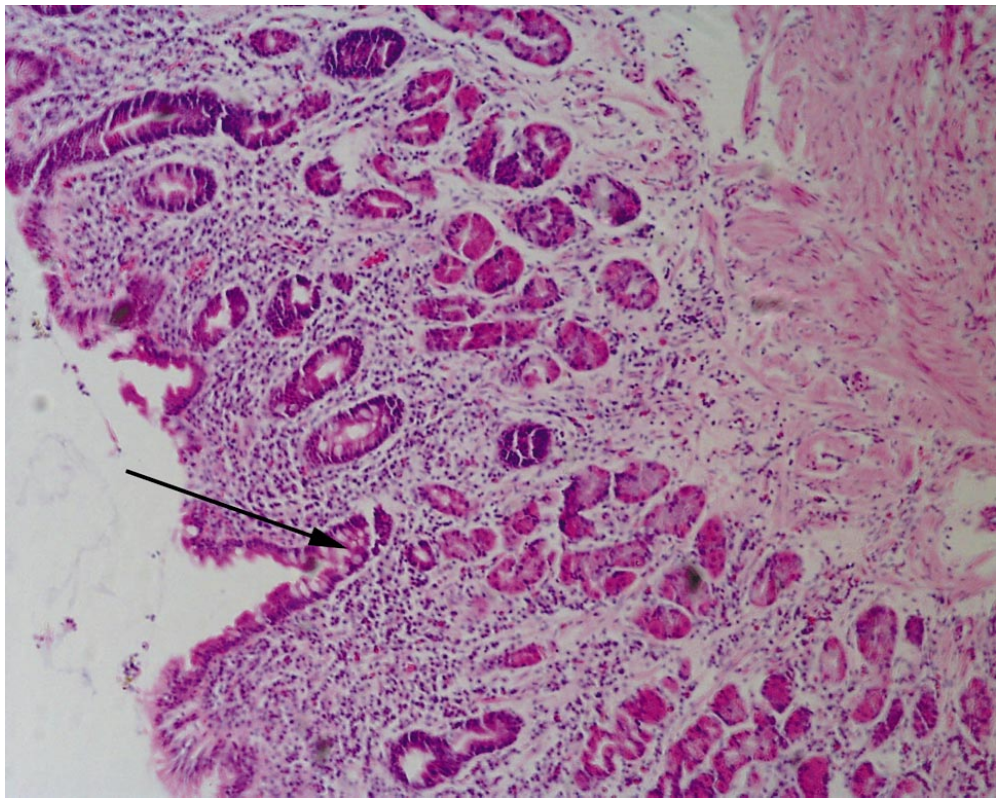

Fig. 1 Atrophic gastritis of moderate degree in the corpus. There is a remarkable loss of oxyntic glands and intestinal metaplasia (*arrow*) is present. Chronic inflammation is mild or moderate, and inactive. HE, original magnification ×100

[21–23] with a high prevalence of *H. pylori* infection [28].

Materials and methods

This study was approved by the Committee of Ethics of the University of Tartu. All persons gave their informed consent prior to their inclusion in the study.

Subjects

Seventy persons (mean age 57.8±11.4; 31 men, 39 women) who had been part of an adult random sample of 304 persons from Saaremaa Island, most of whom had previously been investigated by endoscopy and gastric biopsy in 1979, 1985 and 1991 [25–27], were re-investigated in 1997. In 1997, gastric biopsy specimens from the antrum and corpus mucosa and also serum samples were obtained from 66 of these 70 persons (mean age 57.7±11.3; 30 men, 36 women). Inclusion criteria for 62 (mean age 57.6±11.4, 30 men, 32 women) out of 66 persons for assessment of ACAB were: seropositivity for *H. pylori* in ELISA, presence of *H. pylori* in antrum and/or corpus biopsy specimens assessed by two pathologists.

Additionally, antrum and corpus biopsies and serum samples for 37 out of the 62 persons (mean age 43.7±10.6, 21 men, 16 women) were available from 1985, i.e. from a time 12 years earlier. Serum samples from both points of time were stored at –20°C and were then screened for ACAB and classic PCA.

Formalin-fixed, paraffin-embedded gastric biopsy specimens from the antrum (3 specimens) and corpus mucosa (6 specimens) were stained with haematoxylin and eosin and with a modified Giemsa stain. *H. pylori* colonisation, grade and activity of gastritis, and presence of gastric mucosa atrophy were assessed independently by two pathologists (P.S. and G.F.) in blinded fashion in accordance with the updated Sydney system [3] and scored from 0 (no changes) through 1 (mild) and 2 (moderate) to 3 (severe changes) (Fig. 1).

Anticanalicular autoantibodies

ACAB reacting against canalicular structures within human parietal cells were detected using an immunohistochemical method described earlier [4]. Briefly, heterologous formalin-fixed and paraffin-embedded gastric corpus mucosa with no pathological alterations and not expressing blood groups A and B were incubated overnight at 20°C with sera diluted 1:100 in RPMI 1640 medium (Biochrom, Berlin, Germany). As the secondary antibody, alkaline phosphatase-conjugated rabbit anti-human IgG (DAKO, Hamburg, Germany), diluted 1:10 in RPMI 1640 medium was used (incubation at 20°C for 90 min). After washing, positive reaction was induced with fast red. Sections were counterstained with haematoxylin, mounted with Aquatex and examined by light microscopy, without knowledge of the respective patient data or histological alterations. One positive control serum was included in each immunohistochemical staining session, and negative controls were performed by omitting the human serum. Intensive red immunohistochemical staining, visible at the canaliculi of parietal cells in the corpus mucosa, was taken as a positive reaction (Fig. 2).

Parietal cell antibodies

Classic IgG-type PCA were examined in all sera by an indirect immunofluorescence method, as described previously [21].

H. pylori status

H. pylori status was determined on the basis of a serological evaluation of IgG antibodies to *H. pylori* (strain CCUG 17874), using the enzyme-linked immunosorbent assay (ELISA) method, as re-

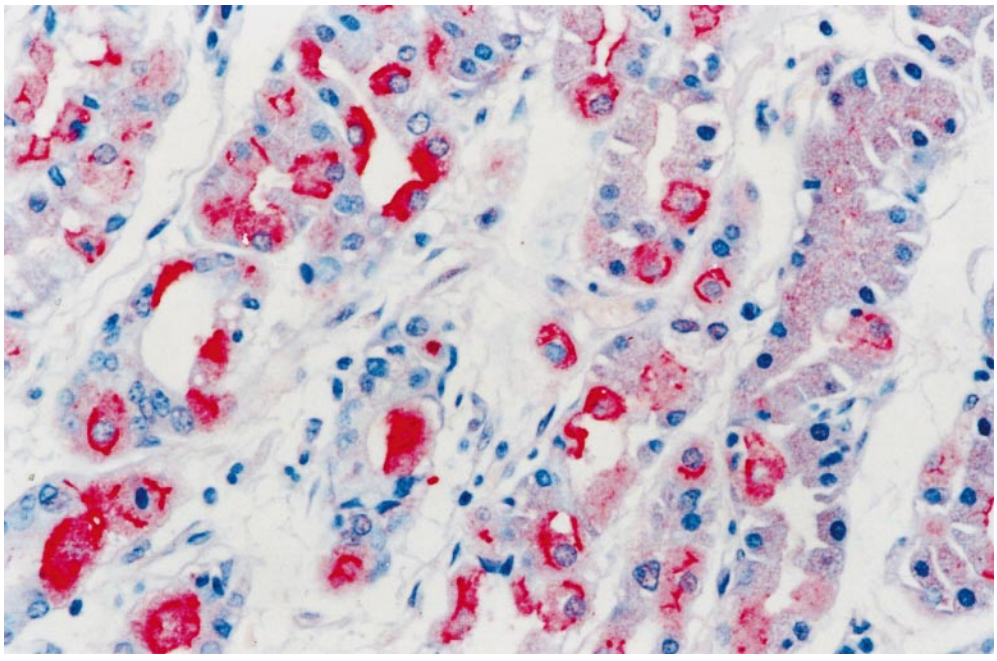

Fig. 2 In situ binding pattern of anticanalicular autoantibodies reacting against canalicular membranes within human parietal cells of the gastric corpus mucosa. Original magnification ×400

ported previously [28]. Briefly, 0.5 µg of acid glycine (pH 2.2)-extracted cell surface proteins per well were used for coating microtitre plates (NUNC, Roskilde, Denmark). The studied sera were diluted to 1:800; the secondary antibody was alkaline phosphatase-labelled anti-human IgG (DAKO, Glostrup, Denmark), which was diluted 1:500. The results were expressed by corrected mean absorbance values as percentages of the reference standard (human gamma globulin, Pharmacia and UpJohn, Stockholm, Sweden). The cut-off value for seropositivity was set at a relative antibody activity (RAA) of 25.

Statistics

Statistical differences between the ACAB-positive and ACAB-negative groups were evaluated by χ^2-and the McNemar tests using Statgraphics and SPSS statistical software. Odds ratios (OR) with 95% confidence intervals (CI) were estimated using the Mantel-Haenzel statistics with the Exact software. Differences were considered statistically significant if 95% CI did not include the value 1.0. Differences in the mean values of age were calculated by the Mann-Whitney test (Statgraphics software). The κ-value for measuring agreement between histological assessments was calculated using SPSS statistical software. *P*-values <0.05 were considered significant.

Results

H. pylori status

In the sample of 1997, positive *H. pylori* serology in ELISA was evaluated in 63 out of the 66 (95%) sera studied. Morphologically, *H. pylori* was confirmed in 62 of them. Three persons were serologically and morphologically negative for *H. pylori*, while 1 was serologically positive but morphologically negative.

The sensitivity of ELISA was 100%, specificity 75%, positive predictive value (PPV) 98%, and negative predictive value (NPV) 100%. Interassay variation was 13% and intra-assay variation was 5%.

In all 37 subjects who passed the follow-up study, *H. pylori* infection was confirmed histologically from the presence of bacteria in biopsy specimens from the antrum and/or corpus as well as from positive *H. pylori* serology in ELISA at both time points.

Gastric mucosa alterations

The grading of chronic inflammation, activity of gastritis, atrophy, intestinal metaplasia and *H. pylori* colonisation in the antrum and corpus mucosa samples in the follow-up group are presented in Table 1.

The grading of antrum and corpus mucosa atrophy, as evaluated independently by two pathologists, is displayed in Table 2. Interobserver agreement regarding atrophy in follow-up samples was as follows: κappa values for samples of 1985: 0.770 (agreement 94.5%) for antrum biopsies and 0.548 (agreement 89.2%) for corpus biopsies; kappa values for samples of 1997: 0.247 (agreement 67%) for antrum biopsies and 0.455 (agreement 73%) for corpus biopsies. Kappa values for 62 samples of 1997 were 0.376 (agreement 72.5%) for antrum biopsies and 0.416 (agreement 71%) for corpus biopsies.

Prevalence of ACAB

Prevalence of ACAB in the follow-up group (samples of 1985 and 1997) and in 62 persons investigated in 1997 are presented in Tables 1 and 3.

ACAB were immunohistochemically detectable in 29 of the 62 (47%) subjects investigated in 1997. In the follow-up group, made up of 37 infected persons, ACAB were detected in 8 of the 37 (22%) in 1985 and in 17 of

Table 1 Grading of chronic inflammation, activity of gastritis, atrophy, intestinal metaplasia, *Helicobacter pylori* and anticanicular autoantibody (*ACAB*) positivity in antrum and corpus mucosa samples in the follow-up group (*n*=37) in 1985 and 1997

Grade	Chronic inflammation		Activity of gastritis		Atrophy		Intestinal metaplasia		*Helicobacter pylori*	
	1985 ACAB+/*n*	1997 ACAB+/*n*	1985 ACAB+/*n*	1997 ACAB+/*n*	1985 ACAB+/*n*	1997 ACAB+/*n*	1985 ACAB+/*n*	1997 ACAB+/*n*	1985 ACAB+/*n*	1997 ACAB+/*n*
In antrum										
0	0/0	0/0	0/1	0/1	5/33	9/23	6/32	12/30	1/4	0/2
1, mild	0/1	0/1	1/2	6/11	2/2	5/11	2/5	5/7	3/14	6/12
2, moderate	6/27	13/29	6/26	11/25	1/2	3/3	0/0	0/0	3/12	1/3
3, severe	2/9	4/7	1/8	0/0	0/0	0/0	0/0	0/0	1/7	10/20
Total	8/37	17/37	8/37	17/37	8/37	17/37	8/37	17/37	8/37	17/37
In corpus										
0	0/0	0/0	1/4	0/0	7/34	10/23	6/33	14/33	1/4	0/2
1, mild	3/11	3/7	3/12	8/19	0/0	5/11	2/4	3/4	4/13	8/12
2, moderate	4/24	11/25	4/20	9/18	1/3	2/3	0/0	0/0	2/15	2/6
3, severe	1/2	3/5	0/1	0/0	0/0	0/0	0/0	0/0	1/5	7/17
Total	8/37	17/37	8/37	17/37	8/37*	17/37**	8/37	17/37	8/37	17/37

Statistically significant difference: **>*P=0.004 (on the basis of McNemar test)

Table 2 Grading of antrum and corpus mucosa atrophy in the samples of 1985 and 1997 as evaluated independently by two pathologists

Grade	Follow-up group				Samples of 1997 (*n*=62)	
	1985 (*n*=37)		1997 (*n*=37)		Pathologist I	Pathologist II
	Pathologist I	Pathologist II	Pathologist I	Pathologist II		
In antrum						
1, mild	3	2	7	11	14	14
2, moderate	3	2	1	3	2	6
3, severe	0	0	0	0	0	0
Total (1–3)	6	4	8	14	16	20
In corpus						
1, mild	4	0	10	11	17	19
2, moderate	2	3	5	3	9	6
3, severe	1	0	3	0	4	1
Total	7	3	18	14	30	26

them (46%) in 1997. All 8 patients who were positive for ACAB in 1985 remained positive for these autoantibodies, whereas 9 of the 29 patients who were negative in 1985 developed ACAB during the follow-up period. The increase in ACAB during this period was statistically significant (P=0.004; McNemar test). These results are presented in Fig. 3.

At the study point in 1985, the mean age (41.7±10.3) of the ACAB-negative persons who were also negative in 1997 did not differ significantly from the mean age (46.1±10.6) of those who were ACAB positive in 1985 or had become ACAB positive in 1997 (P=0.18).

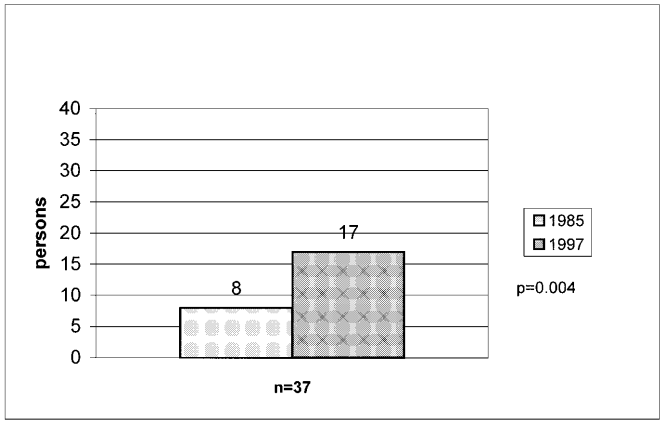

Fig. 3 Significant increase in anticanalicular autoantibodies in a 12-year follow-up of untreated *H. pylori* gastritis

Prevalence of PCA

Among the 62 serum samples examined in 1997, 3 were positive for classic PCA. All these 3 persons were also positive for ACAB in 1997. In the follow-up sample, all 37 patients were negative for classic PCA in 1985, while

Table 3 Grading of chronic inflammation, activity of gastritis, atrophy, intestinal metaplasia, *Helicobacter pylori*, as evaluated by two pathologists, and ACAB positivity in antrum and corpus mucosa samples in a group of 62 persons in 1997

Grade	Chronic inflammation		Activity of gastritis		Atrophy			Intestinal metaplasia		*Helicobacter pylori*	
	Pathologist		Pathologist		Pathologist			Pathologist		Pathologist	
	I ACAB+/*n*	II ACAB+/*n*	I ACAB+/*n*	II ACAB+/*n*	I ACAB+/*n*	II ACAB+/*n*		I ACAB+/*n*	II ACAB+/*n*	I ACAB+/*n*	II ACAB+/*n*
In antrum											
0	1/2	0/0	4/10	0/1	20/46	18/42		21/50	22/49	2/3	2/3
1, mild	3/9	0/0	22/47	10/19	8/14	7/14		6/9	7/13	11/18	11/18
2, moderate	19/40	20/48	3/5	19/41	1/2	4/6		2/3	0/0	3/9	3/9
3, severe	6/11	9/14	0/0	0/1	0/0	0/0		0/0	0/0	13/32	13/32
Total	29/62	29/62	29/62	29/62	29/62	29/62		29/62	29/62	29/62	29/62
In corpus											
0	0/1	0/0	7/20	0/1	10/32*	13/36†		21/54	20/49	0/0	0/0
1, mild	3/10	3/10	13/29	14/30	7/17	10/19		7/7	9/13	14/21	14/21
2, moderate	13/29	18/41	9/13	15/30	8/9	5/6	††	1/1	0/0	3/10	3/10
3, severe	13/22	8/11	0/0	0/1	4/4	1/1		0/0	0/0	12/31	12/31
Total	29/62	29/62	29/62	29/62	29/62	29/62		29/62	29/62	29/62	29/62

Statistically significant differences: **>*P=0.01; ††>†P=0.04; > P=0.004 (on the basis of the Chi-square test)

2 of the 37 had become PCA positive in 1997. The latter were also ACAB positive in 1985 and 1997. All PCA-positive persons revealed highly intensive staining for ACAB throughout the cytoplasm.

Among the persons who possessed PCA in 1997, 1 had grade 2, and another had grade 3 corpus atrophy, while the third showed grade 2 chronic inflammation in the corpus but no atrophy. In the follow-up group, of the 2 persons who developed PCA in 1997, 1 developed grade 2 corpus atrophy, but the other did not. The latter person had grade 2 chronic inflammation in the corpus at both time points.

Course of gastritis and ACAB

In samples for 62 persons examined in 1997 a significant association was found between ACAB and corpus mucosa atrophy (grades 1–3) by both pathologists. According to pathologist I, of 30 persons with corpus mucosa atrophy (grades 1–3), 19 (63%) had ACAB, while autoantibodies were found in only 10 of the 32 (31%) subjects without atrophy (P=0.01; OR=3.8; 95% CI 1.4–10.6). According to the diagnosis of atrophy established by pathologist II, 16 of 26 (62%) subjects with corpus atrophy grade 1–3 had ACAB, whereas only 13 of 36 (36%) persons without corpus atrophy had these autoantibodies (P=0.04; OR=2.8; 95% CI 1.02–7.8).

In Table 4 the relationship between ACAB and development of corpus atrophy of different grades is shown separately for 37 persons who were followed up for 12 years. In summary, according to pathologist I (the values given by pathologist II are presented in brackets) 2 (1) of 8 patients with ACAB had some grade of atrophy in 1985, while 5 (2) of 29 infected patients without ACAB also had corpus atrophy already in 1985 (P=1.0). In 1997, 10 (7) out of 17 ACAB-positive subjects had atrophy (grade 1, 4; grade 2, 4; grade 3, 2; according to pathologist I; grade 1, 5; grade 2, 2; according to pathologist II), while 8 (7) out of 20 ACAB-negative patients showed similar mucosal alterations (atrophy grade 1, 6; grade 2, 2 according to pathologist I; atrophy grade 1, 6 grade 2, 1; according to pathologist II; P=0.25). Thus, although the prevalence of atrophy determined by either pathologist was higher in the subset of infected persons with ACAB than in the subset without these autoantibodies, no statistically significant association between ACAB and corpus mucosa atrophy could be established either at the beginning of the study or at the end of the follow-up period.

Furthermore, out of 9 persons who developed ACAB during the follow-up period, 4 (3) subjects also developed corpus mucosa atrophy (grade 1, 2; grade 2, 1; grade 3, 1 according to pathologist I; grade 1, 2; grade 2, 1 according to pathologist II). On the other hand, out of the 14 (11) persons who developed atrophy (grade 1 , 9; grade 2, 1; grade 3, 1 according to pathologist I; grade 1, 9; grade 2, 2 according to pathologist II) in the follow-up period, 6 (6) had no autoantibodies either at the begin-

Table 4 Grading of corpus atrophy in persons with and without ACAB in 1985 and 1997 (*n*=37). Atrophy was graded from 0 (no atrophy) through 1 (mild) and 2 (moderate) to 3 (severe)

Pat. no.	ACAB in 1985	ACAB in 1997	Pathologist I		Pathologist II	
			Corpus atrophy 1985	Corpus atrophy 1997	Corpus atrophy 1985	Corpus atrophy 1997
1	–	–	0	1	0	0
2	–	–	0	1	0	1
3	–	–	2	0	2	1
4	–	–	0	0	0	0
5	–	–	0	2	0	2
6	–	–	0	0	0	0
7	–	–	0	0	0	0
8	–	–	0	0	0	0
9	–	–	0	0	0	1
10	–	–	0	0	0	0
11	–	–	0	0	0	0
12	–	–	1	2	0	1
13	–	–	0	0	0	0
14	–	–	0	1	0	0
15	–	–	0	1	0	0
16	–	–	1	0	0	1
17	–	–	0	1	0	0
18	–	–	0	0	0	0
19	–	–	1	1	0	1
20	–	–	0	0	0	0
21	–	+	0	1	0	1
22	–	+	0	3	0	2
23	–	+	0	0	0	0
24	–	+	0	1	0	0
25	–	+	0	2	0	1
26	–	+	0	0	0	0
27	–	+	3	2	2	2
28	–	+	0	0	0	0
29	–	+	0	0	0	0
30	+	+	0	2	0	1
31	+	+	0	0	0	0
32	+	+	0	1	0	0
33	+	+	2	3	2	1
34	+	+	0	1	0	0
35	+	+	0	2	0	1
36	+	+	1	0	0	0
37	+	+	0	0	0	0

ning or at the end of the study (P=1.0). Again, at follow-up no statistically significant correlation was found between evolution of ACAB and development of gastric atrophy, or vice versa.

Intestinal metaplasia and ACAB

The grading of intestinal metaplasia and its association with ACAB are presented in Tables 1 and 3. In the follow-up group, persons with corpus intestinal metaplasia grade 1 were ACAB positive in 2 out of 4 cases in 1985 and in 3 out of 4 in 1997 (P>0.05); persons with antrum intestinal metaplasia grade 1 were ACAB+ in 2 out of 5 cases in 1985 and in 5 out of 7 cases in 1997 (P>0.05). In the larger sample of 1997 (n=62) a significant association was noted between presence of intestinal metaplasia in the corpus (grades 1–2) and ACAB (8 ACAB positive out of 8 cases with intestinal metaplasia grade 1–2 versus 21 ACAB positive out of 54 cases without intestinal metaplasia; P=0.004). This association was not significant in the antrum mucosa: 8 ACAB positive out of 12 cases with intestinal metaplasia versus 21 out of 50 cases without intestinal metaplasia (P=0.22).

Isolated antrum atrophy and ACAB

With reference to the association between atrophic antrum gastritis and ACAB, cases with isolated atrophic antrum gastritis are of special interest. In the samples of 1997 (n=62), according to the data of pathologist I there were 4 cases of isolated antrum atrophy, while 2 of them were ACAB positive. According to the data of pathologist II, 4 out of 9 cases of isolated antrum atrophy were ACAB positive. In the follow-up group, according to the data of pathologist I, 2 persons developed isolated antrum atrophy (1 ACAB positive); according to the data of pathologist II, 4 persons developed isolated antrum atrophy (2 of them ACAB positive).

Chronic inflammation, activity of gastritis, *H. pylori* colonisation and ACAB

ACAB positivity at different grades of chronic inflammation and activity of gastritis and at different grades of *H. pylori* colonisation are presented in Tables 1 and 3. The tendency to an increase in the prevalence of ACAB in 1997 compared with 1985 was seen in a group of persons with grade 2 chronic inflammation in corpus mucosa (11/25 versus 4/24; P=0.14, McNemar test). During the follow-up period ACAB formed in 9 of the 37 persons studied. Among these 9 persons the grade of chronic inflammation in the corpus mucosa progressed in 5 cases, retained the same in 3 cases and decreased in 1 case. The grade of chronic inflammation in the antrum mucosa progressed in 2 cases, remained the same in 6 cases and decreased in 1 case.

In the corpus mucosa, periglandular lympocytic infiltrates were found in the samples of 1985 in 5 of the 37 cases, while all these 5 cases were ACAB negative both in 1985 and in 1997. In the 1997 sample periglandular lymphocytic infiltrates were found in 9 cases (7 of them newly diagnosed) and ACAB were present in 4 out of 9 cases. All persons with the finding of periglandular lymphocytic infiltrates were PCA negative.

Concerning changes in the activity of corpus gastritis in persons who developed ACAB, the grade of activity progressed in only 1 case, remained the same in 6 cases and decreased in 2 cases. In the antrum mucosa no progression of activity was observed, activity remaining the same grade in 4 cases and decreasing in 5 cases.

Thus, there was no significant association between grade of activity of gastritis and the presence or absence of ACAB at any time of observation. A tendency to an increase in the prevalence of ACAB during 12 years' follow-up was observed among persons with moderate grades of chronic inflammation.

In the sample of 1997 the number of persons with *H. pylori* colonisation grade 3 had increased significantly: in antrum specimens from 7 of 37 in 1985 to 20 of the 37 in 1997 (P=0.003); in corpus specimens from 5 of the 37 in 1985 to 17 in 1997 (P=0.005). However, development of ACAB in these persons was not statistically significant (P=0.11 for antrum specimens with *H. pylori* colonisation grade 3; P=0.42 for corpus mucosa specimens with *H. pylori* colonisation grade 3) and only 1 person in whom the grade of *H. pylori* colonisation progressed to severe developed a severe grade of atrophy in the corpus.

Discussion

The present study represents the first prospective investigation of anticanalicular autoantibodies (ACAB) and their significance for development of atrophic gastritis in a long-term follow-up of an infected random adult cohort with noneradicated *H. pylori*. In a previous study by Uibo et al. [22] the correlation between classic anti-PCA and development and progression of chronic gastritis was studied in a similar group followed up for 6 years. Furthermore, a 32-year follow-up study of chronic *H. pylori* gastritis has provided evidence that progression of corpus gastritis might be associated with the occurrence of PCA [24].

The most important finding of the present study was that the prevalence of ACAB increased significantly during the follow-up period of 12 years. One might suppose that ACAB are formed in the course of time and their appearance may be related to the duration of *H. pylori* infection. However, as the exact time of onset of *H. pylori* infection is unknown in the persons enrolled in this study, the duration of infection cannot be established either. It should be pointed out that the mean age of persons who were positive for ACAB at the beginning of the study, or became positive for ACAB during the next 12 years, did not differ significantly from the mean age of those who remained negative for ACAB during the whole period. This is in agreement with earlier data published by Faller et al. [5], indicating that the age profile of *H. pylori*-infected patients with antigastric autoantibodies was not significantly different from the age profile of patients without these autoantibodies. As most *H. pylori* infections occur in childhood [19] and can persist throughout life, the age of an infected adult person and duration of *H. pylori* infection can be regarded as roughly equivalent. The evidence of the role of the person's age or duration of *H. pylori* gastritis in formation of ACAB is based on studies of infected children, in which a lower prevalence of ACAB was reported [7, 9]. Furthermore, a significant age-dependent increase in PCA positivity was shown in *H. pylori*-infected persons but not in *H. pylori*-negative persons, which suggests that long-lasting *H. pylori* gastritis might represent an important factor for formation of PCA [23]. Other studies in which the formation of classic PCA was followed up over time have given similar results [8, 22].

The association of ACAB with *H. pylori* gastritis, particularly with atrophic gastritis, in the corpus mucosa has been established in several previous studies [2, 4, 5, 15, 20] and was confirmed again in a group of 62 persons in this investigation. The histological diagnosis of atrophy is difficult, and significant interobserver variation in the determination and grading of atrophy has been reported only recently [16]. The two pathologists who participated in this study attained interobserver agreement comparable to that seen in the last study. The fact that both investigators found a significant correlation between corpus atrophy and ACAB confirms the existence of a link between antigastric autoimmunity and gastric atrophy in *H. pylori* gastritis.

Progression of superficial gastritis to atrophic gastritis has been shown in several previous follow-up studies [10, 11, 24–27]. In the present study we attempted to find an answer to the question of why gastritis remains confined to superficial mucosal layers in some patients while gastric atrophy develops in others. Since ACAB are correlated with atrophic corpus gastritis, and an autoimmunological reaction can lead to loss of parietal cells and to evolution of gastric atrophy, then ACAB can

serve as relevant markers for defining patients who are at higher risk for development of gastric atrophy [5]. However, in this follow-up study we could not establish a significant association between presence of ACAB and development of atrophy over the 12 study years. The lack of any such correlation is obviously due to the low number of enrolled persons, since such a correlation was observed when the number of patients with the same epidemiological background was increased from 37 to 62 at the end of the study. Also, the study period might have been too short. Uibo et al. (1989) reported that in a 6-year follow-up period, changes in the gastric mucosa in autoimmune gastritis and in autoantibody-negative gastritis revealed no significant differences [22]. The antrum and corpus mucosa of 2 persons who developed PCA did not reveal any alterations over 6 years. In contrast, in a much longer, 32-year, follow-up study, the appearance of PCA occurred in parallel with progression of corpus atrophy, disappearance of *H. pylori* and improvement of the antral mucosa [24].

Although our study could not establish a significant correlation between ACAB and development of atrophic alterations in the corpus mucosa, we found a tendency to an increase in the prevalence of ACAB over the 12 study years in persons with moderate chronic lympocytic infiltration in the corpus mucosa and in the case of intestinal metaplasia in the corpus. The importance of lymphocytic infiltration in the corpus mucosa, especially in association with intestinal metaplasia, as an increased risk for development of gastric carcinoma, was stressed in a study conducted by Meining et al. [13].

As in previous investigations, all PCA-positive persons in our study were also positive for ACAB [5]. On the other hand, only 3 of all 29 ACAB-positive persons were positive for PCA. This can be explained by different binding sites of ACAB and classic PCA within parietal cells [2]. Interestingly, 3 persons who had classic PCA in 1997 showed very intensive staining for ACAB throughout the cytoplasm. Two of them had moderate or severe corpus atrophy. In the follow-up group only 2 persons turned out to be positive for PCA in 1997. However, although both had already revealed intensive staining for ACAB in 1985 and 1997, 1 developed corpus atrophy but the other did not. It could be hypothesised that ACAB precede classic PCA and that epitope switching takes place in the process of *H. pylori*-associated antigastric autoimmunity. This hypothesis could also explain why not all ACAB-positive persons were PCA positive in our study and in previous research [2, 5].

Our study confirmed previous reports of a significant correlation between ACAB and gastric mucosa atrophy [5, 6, 20]. However, from the data presented in this study we cannot clearly define whether ACAB are the cause or the consequence of gastric atrophy. There are some patients who are ACAB positive but (still) do not have atrophy. In these patients antigastric autoimmunity precedes atrophy. On the other hand, there are also patients who have atrophy but who are negative for ACAB. We can surmise that in this subgroup of persons ACAB might be developed during further progression of atrophy and appear when gastric cells are sufficiently degraded and autoantigens are presented to immunocompetent cells. It is evident that further research into antigastric autoimmunity is needed to get a better insight in the pathogenesis of chronic atrophic gastritis.

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