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Apoptosis in chronic gastritis and its correlation with antigastric autoantibodies

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Abstract In the course of time, chronic gastritis may result in gastric atrophy, as in type A gastritis, where autoimmune reactions against parietal cells result in a loss of corpus glands. Two antigastric autoantibodies have been detected in *Helicobacter pylori* gastritis and are described as anti-luminal and anti-canalicular autoantibodies. The aim of this study was to determine whether increased apoptosis may be responsible for the loss of gastric epithelium and whether this apoptosis is correlated with antigastric autoimmunity. Gastric biopsies from normal mucosa and *Helicobacter pylori* gastritis were analysed for the presence of apoptosis using the TUNEL method. *Helicobacter pylori* gastritis was divided into cases (1) without autoantibodies, (2) with anti-luminal, and (3) with anti-canalicular autoantibodies. Apoptotic cells of the foveolar and of the glandular epithelium in the antrum and corpus were counted. The number of apoptotic cells in the gastric mucosa was significantly increased in all cases of gastritis. The highest number of apoptotic cells was observed in the gastric glands of the corpus mucosa in *Helicobacter pylori* gastritis with anti-canalicular autoantibodies. Apoptosis contributes to the development of gastric atrophy and there are various types of *Helicobacter pylori* gastritis. The positive correlation between apoptotic cell loss in the glandular zone of the corpus mucosa and the presence of anti-canalicular autoantibodies indicates a possible link between antigastric autoimmunity and atrophy in this type of *Helicobacter pylori* gastritis – similar to that in classic type A gastritis.

Key words *Helicobacter pylori* · Anti-gastric autoantibodies · Apoptosis · Gastric atrophy

Dedicated to Prof. Dr. Volker Becker on the occasion of his 75th birthday

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Introduction

Long-term *Helicobacter pylori* (Hp) gastritis can be complicated by glandular atrophy and intestinal metaplasia [17, 18, 20]. Atrophy and intestinal metaplasia probably represent the pathogenic link between Hp infection and gastric carcinogenesis [14, 24, 25].

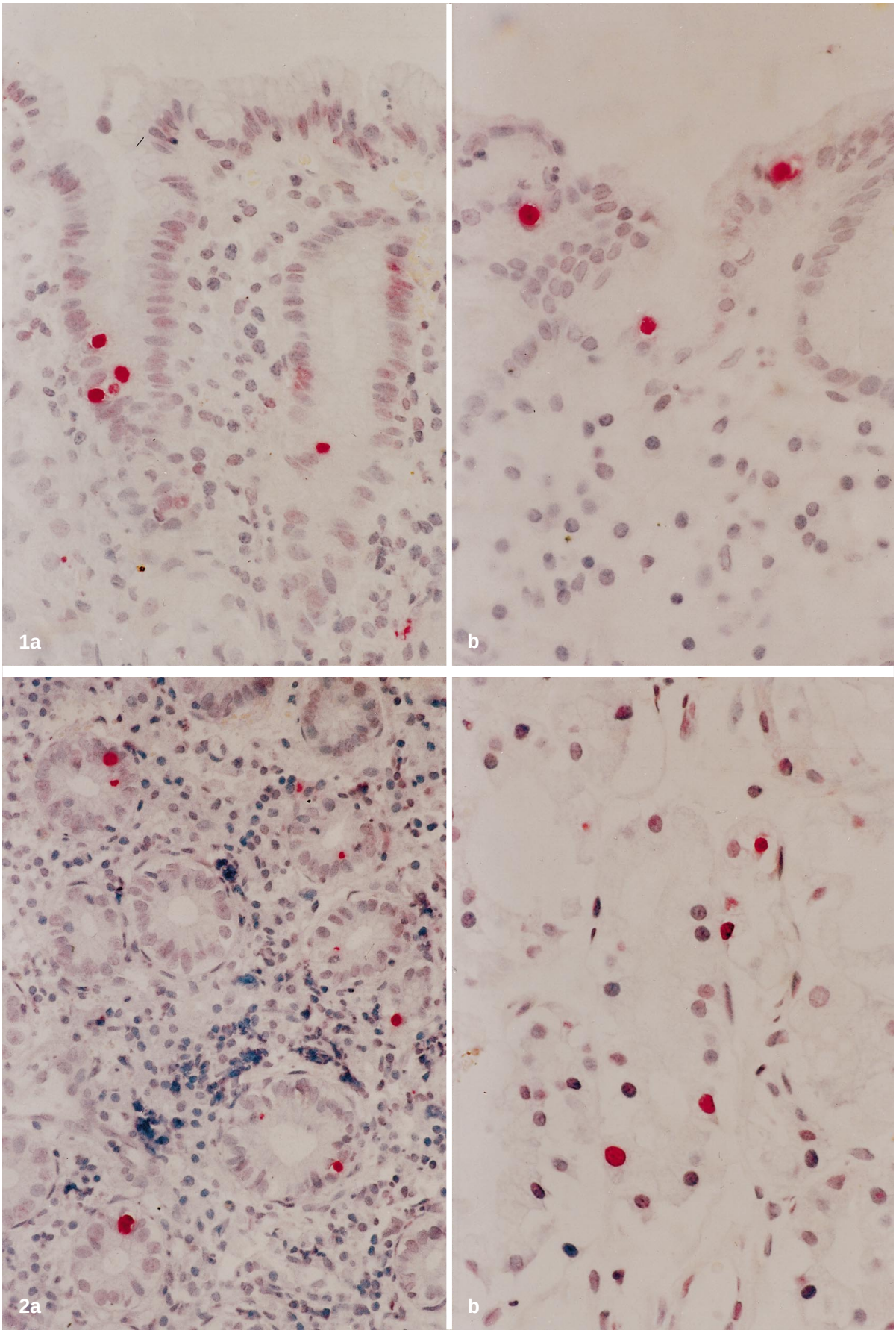
Corpus and fundus atrophy in type A gastritis is the consequence of an antigastric autoimmune reaction with the presence of autoantibodies against parietal cells [26]. Anti-gastric autoantibodies have also been detected in Hp gastritis [22]. Recently, we found that anti-gastric autoantibodies occur in up to 50% of Hp-infected patients and that there are two distinct binding patterns of the autoantibodies [6–8, 15]: anti-luminal autoantibodies react against the luminal membranes of the foveolar epithelium and anti-canalicular autoantibodies bind to the canaliculi within human parietal cells. The presence of anti-canalicular serum autoantibodies correlates with the severity of body gastritis and with gastric corpus atrophy in patients with Hp gastritis [6–8, 23]. Since increased apoptosis is a well-known mechanism of cell loss in inflammatory reactions and autoimmune diseases [16, 19, 21], we evaluated apoptosis in situ in the foveolar and glandular epithelial compartment in normal gastric mucosa and in Hp gastritis, with and without anti-gastric autoantibodies.

Materials and Methods

Paraffin-embedded and rountinely processed biopsies of the antrum and corpus of 47 patients (age 25–87 years) were investigated (Table 1). The material of the study comprised 12 cases with normal mucosa and 35 cases of Hp gastritis.

Depending on the presence of anti-gastric autoantibodies with anti-luminal or anti-canalicular specificities, Hp gastritis was divided (Table 1) into cases without anti-gastric autoantibodies (n=16), cases with anti-luminal autoantibodies (n=7) and cases with anti-canalicular autoantibodies (n=12). Antibodies to Hp had been ascertained by ELISA and anti-gastric autoantibodies, by immunohistochemistry [6].

Sections, 4 μ thick, were cut from paraffin-wax-embedded tissue blocks. The updated Sydney system was used for classification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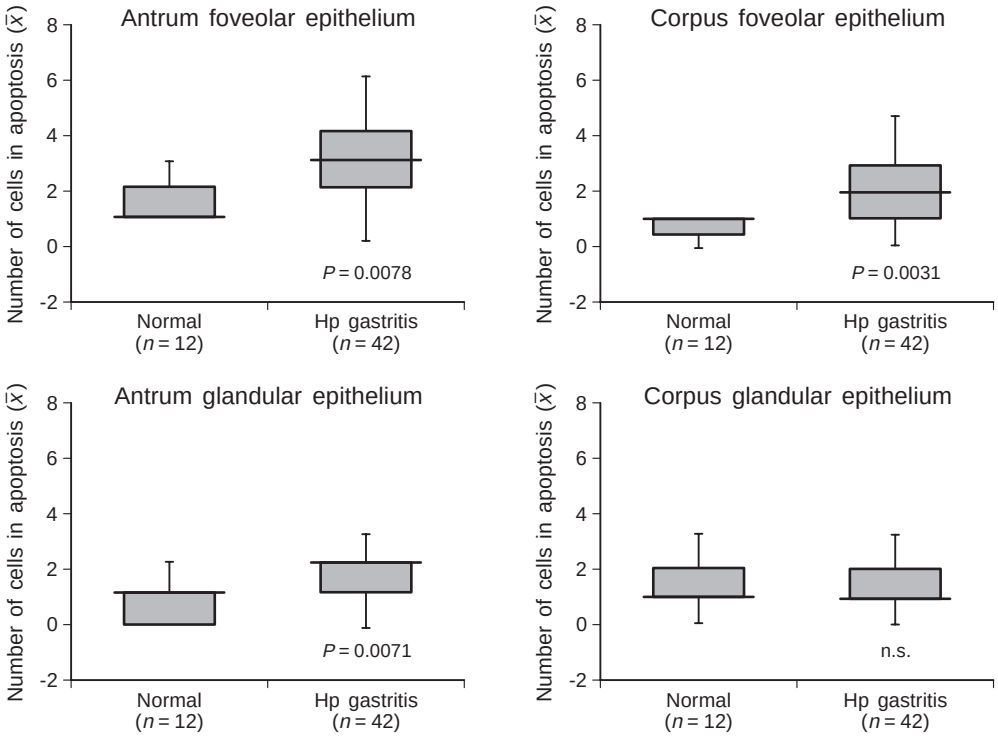


Fig. 3 Box plot chart (50% of values within the box. Horizontal bar: median. Vertical bar: range of values): apoptosis of the antral and corpus gastric epithelium in normal mucosa and Hp-gastritis, n. s.=not significan

Table 1 Material investigated in the study (*Hp: Helicobacter pylo*

Materials	*n*
Normal mucosa	12
Hp gastritis	35
	47

Hp gastritis	*n*
Without autoantibodies [NAB]	16
With anti-luminal autoantibodies [LUM AB]	7
With anti-canalicular autoantibodies [CAN AB]	12
	35

and grading of gastritis [4], and Warthin-Starry silver impregnation served for detection of Hp.

Apoptosis was demonstrated using TUNEL (terminal desoxynucleotidyl-transferase mediated dUTP-biotin nick end labelling) histochemistry [9]. Sections were dewaxed, rehydrated and digested with proteinase K (20 µg/ml, Sigma) for 15 min at room temperature. After washing with destilled water and PBS an equilibration buffer (Oncor, Gaithersburg USA) was applied for 5 min at room temperature. The TUNEL reaction was then performed using the ApopTag-Kit (Oncor). By an enzymatic reaction (incubation time 60 min at 37°C) with tdT, dUTP is specifically transferred to the 3' OH ends of DNA. The reaction was finished with a "stop-wash buffer" and sections were subsequently incubated with a fluorescein-labelled antibody against digoxigenin (30 min at room temperature). After washing with PBS and TRIS buffer a monoclonal mouse antifluorescein antibody was added and sections were stained according to the APAAP method using fast red as a substrate. Counterstaining was done with haematoxylin. Apoptotic cells showed a distinct signal in the nucleus (Figs. 1, 2). For negative controls tdT was omitted, resulting in uniformly negative staining. For positive controls, sections were digested with proteinase K and pretreated with DNAse I for 12 min at 37°C, resulting in positive staining of all cells as previously described [9]. Tonsils with germinal centres containing multiple apoptotic cells were used as control tissue.

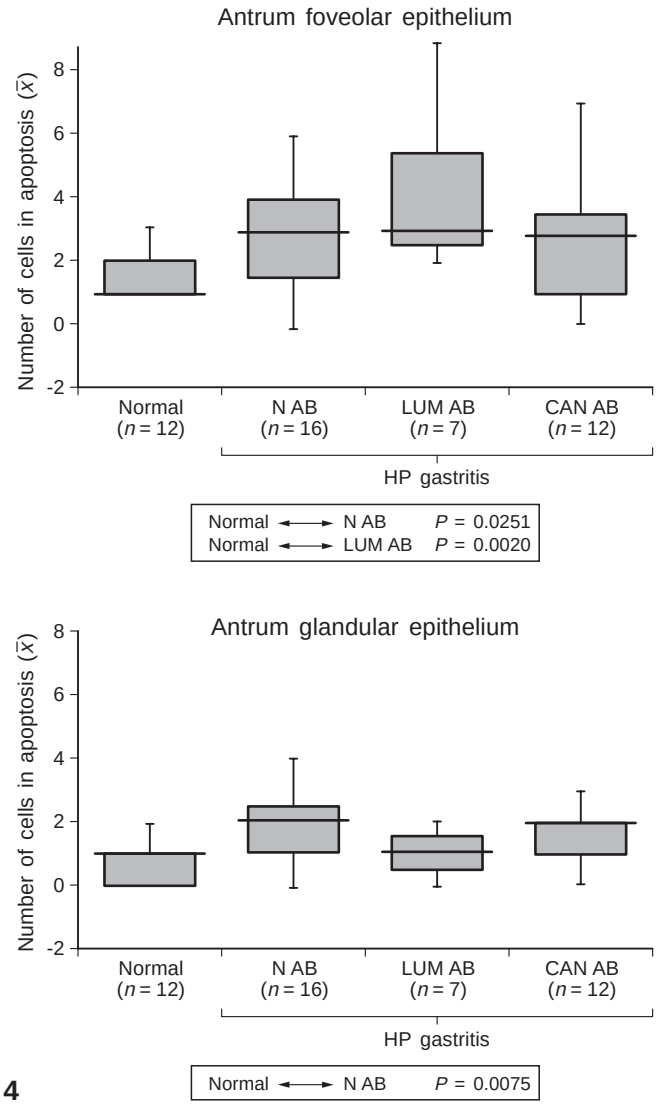
Intraepithelial T-lymphocytes, which can undergo apoptotis and be confused with apoptotic epithelial cells, were stained with CD 43 (MT1, DAKO, Glostrup Denmark) by the APAAP method. Signals that did not allow a clear distinction between an apoptotic lymphocyte and an apoptotic epithelial cell were not scored. The rate of apoptotic cells in the antrum and in the corpus mucosa was quantified by counting 200 cells of the foveolar epithelium and 200 cells of the glandular epithelium. Statistical analysis was performed by means of the Mann-Whitney U-test. A *P*-value smaller than 0.05 was considered significant.

◀ **Fig. 1** Apoptosis of the antral (*left*) and corpus (*right*) foveolar epithelium in cases of Hp (*Helicobacter pylori*) gastritis. TUNEL reaction, haematoxylin counter-staining, ×800

Fig. 2 Apoptosis of the antral (*left*) and corpus (*right*) glandular epithelium in a case of Hp gastritis without autoantibodies (*left*) and with anti-canalicular autoantibodies (*right*). TUNEL reaction, haematoxylin counterstaining, ×800, ×1000

Results

In the normal mucosa few cells in apoptosis were detectable in the foveolar and glandular epithelium. Hp gastritis (Fig. 3) showed a significantly higher number of apoptotic cells in the foveolar epithelium of the antrum and corpus as well as in the glandular epithelium of the antrum. In corpus glandular epithelium the number of apoptotic cells was again elevated, but this increase was statistically not significant compared with normal mucosa (*P*=0.597).



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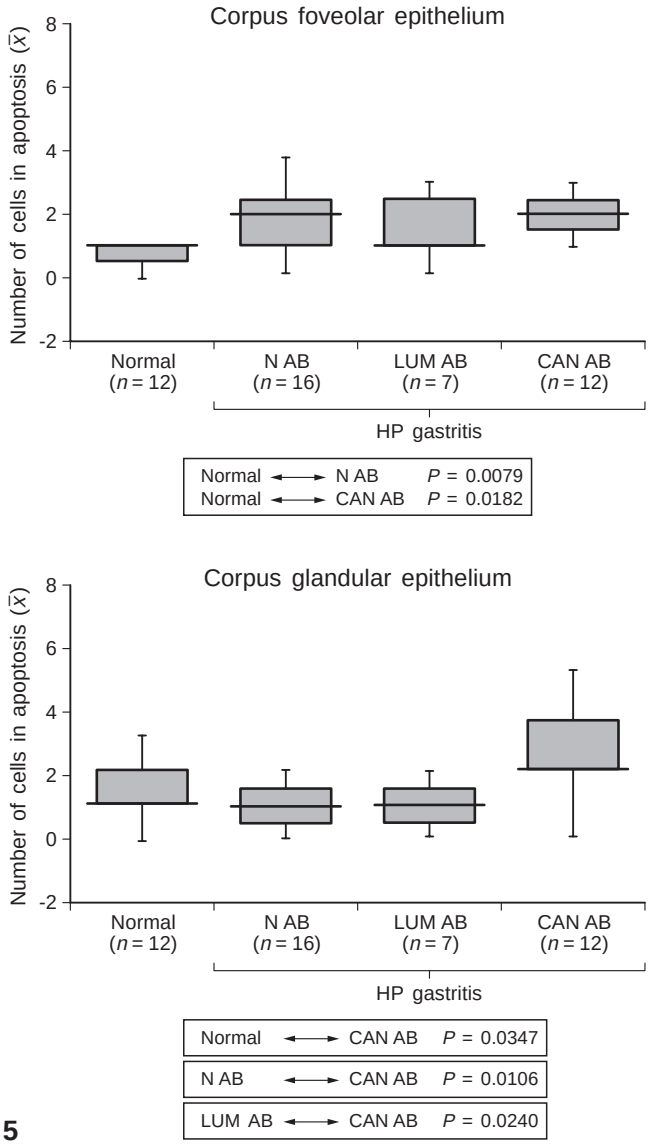

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Figs. 4, 5 Box plot chart (50% of values within the box; *horizontal bar:* median, *vertical bar:* range of values): apoptosis of the antral and corpus gastric epithelium in normal mucosa and different types of Hp gastritis (*N AB:* without antigastric autoantibodies, *LUM AB:* with anti-luminal autoantibodies, *CAN AB:* with anti-canalicular autoantibodies

Subdividing Hp gastritis (Table 1) according to the presence of anti-luminal and anti-canalicular autoantibodies showed that in the foveolar epithelium of the antrum the highest apoptotic rates are found in groups without autoantibodies and with anti-luminal autoantibodies (Fig. 4). Differences between these two groups and normal mucosa were significant. In the group with anti-canalicular autoantibodies the number of apoptotic cells in the foveolar epithelium was also elevated, but without statistical significance (P=0.1196). The glandular epithelium of the antrum had the highest values in the group without autoantibodies followed by the groups with anti-canalicular autoantibodies (P=0.0516) and anti-luminal autoantibodies (P=0.5846). The difference from normal mucosa was significant only in the group without autoantibodies (Fig. 4).

The foveolar epithelium of the corpus (Fig. 5) revealed the highest number of apoptotic cells in the group without autoantibodies and that with anti-canalicular autoantibodies. There was a difference between normal mucosa and the group with anti-luminal autoantibodies, but this was not significant (P=0.1329). In the glandular epithelium of the corpus the highest number of apoptotic cells was found in the group with anti-canalicular autoantibodies (Fig. 5). This increase was significant compared with the levels in all other groups. The differences between normal mucosa and the groups without autoantibodies and with anti-luminal autoantibodies were not significant (P=0.5423 and P=0.5629). In cases with anti-canalicular autoantibodies, histology showed a loose periglandular, lymphocytic infiltrate, which seemed to destroy the corpus glands (Fig. 6).

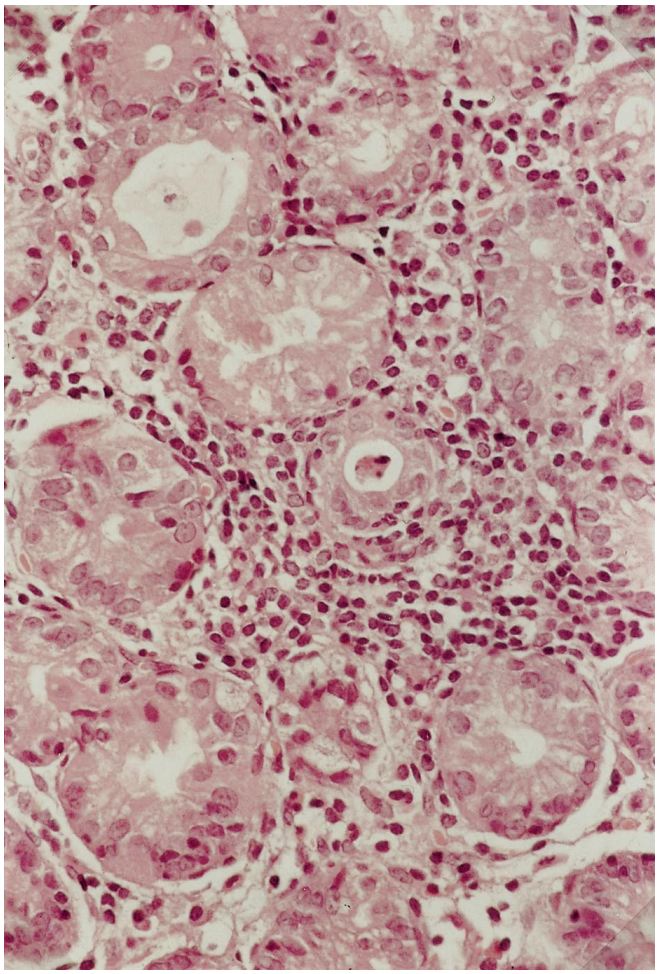

Fig. 6 Periglandular lymphocytic infiltrate of the corpus mucosa in a case with anti-canalicular autoantibodies. HE, ×600

Discussion

The physiological loss of cells in the gastrointestinal tract is mainly regulated by apoptosis [11, 13]. Increased apoptotis of epithelial cells in Hp gastritis has been documented by a previous study [21]; this apoptotic cell loss may be due to virulence factors of the bacteria, to inflammatory cytokines released by neutrophils and macrophages, or to cytotoxic effects of T cells and natural killer cells [21, 28].

Bacterial colonization and inflammation in Hp-gastritis is usually predominant in the antrum and mainly affects the superficial foveolar epithelium [20]. In type A gastritis, in contrast, inflammation is confined to the glandular epithelium of the corpus.

Autoimmune reactions are known to produce glandular atrophy in type A gastritis, but have rarely been discussed in the pathogenesis of Hp gastritis. Our findings [6, 8] and the findings of other groups [1, 22, 23] show that anti-gastric autoimmunity is also present in a considerable proportion of patients with Hp gastritis.

We have demonstrated epithelial apoptotis of the foveolar and glandular compartment in the antrum and corpus in normal mucosa and in Hp gastritis with and without anti-gastric autoantibodies. Hp gastritis in general causes an increase of apoptosis in the foveolar and glandular epithelial cells. With regard to the two subtypes of anti-gastric autoantibodies, anti-luminal and anti-canalicular, there are rather inconspicious differences in the antrum foveolar and glandular epithelium and also in the corpus foveolar epithelium. However, in Hp gastritis with serum autoantibodies against the canaliculi of gastric parietal cells a significantly increased number of apoptotic cells in the glands of the corpus mucosa can be found. The histopathology is quite characteristic, showing a loose periglandular lymphocytic infiltrate in the corpus (Fig. 6). Similar morphological changes have been described in “preatrophic” or “active” type A gastritis [5, 27]. Apoptosis of glandular epithelial cells is a possible mechanism leading to corpus atrophy in type A gastritis – comparable to the loss of cells in other autoimmune diseases, such as Hashimoto thyreoiditis [16].

The presence of anti-canalicular autoantibodies correlates significantly with glandular atrophy, increased fasting gastrin levels and a decrease of the pepsinogen I/II ratio in Hp gastritis [7, 8]. Such correlations are not found in gastritis with anti-luminal autoantibodies, which do not influence the rate of apoptosis compared with Hp gastritis in general.

The multifocal atrophic gastritis in the classification of Correa [3] might be considered a transient form of bacterial and autoimmune pathogenesis. Our results indicate that in some cases of Hp gastritis such pathogenic links to autoimmune gastritis exist. In previous studies serological footprints of Hp infection in patients with type A gastritis or pernicious anaemia have been reported [12], and there have been speculations about combined type A/B gastritis in the past [10].

The functional relevance of the anti-gastric autoantibodies as inducers of glandular apoptosis is not clear in either Hp or type A gastritis. It seems likely that cytotoxic T-cell-mediated autoimmune mechanisms cause apoptotic cell loss in situ [2, 21]. However, anti-gastric autoantibodies may be indicators of an autoimmune process that is decisive for the outcome of Hp infection.

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References

1. Appelmelk BJ, Simoons-Smit I, Negrini R, Moran AP, Aspinall GO, Forte JG, de Vries T, Quan H, Verboom T, Maaskant JJ, Ghiara P, Kuipers EJ, Bloemena E, Tadema T, Townsend RR, Tyagarajyn K, Crothers JM, Monteiro MA, Savio A, de Graaf J (1996) Potential role of molecular mimicry between *Helicobacter pylori* lipopolysaccharide and host Lewis blood group antigens in autoimmunity. Infect Immun 64:2031–2040
2. Bosman FT, Visser BC, van Oeveren J (1996) Apoptosis: pathophysiology of programmed cell death. Path Res Pract 192:676–683
3. Correa P (1980) The epidemiology and pathogenesis of chronic gastritis: three etiologic entities. Front Gastrointest Res 6:98–108

4. Dixon F, Genta M, Yardley JH, Correa P, et al (1996) Classification of gastritis. The updated Sydney system. Am J Surg Pathol 20:1161–1181
5. Eidt S, Oberhuber G, Schneider A, Stolte M (1996) The histopathological spectrum of type A gastritis. Path Res Pract 192:101–106
6. Faller G, Steininger H, Eck M, Hensen J, Hahn EG, Kirchner T (1996) Antigastric autoantibodies in *Helicobacter pylori* gastritis: prevalence, in situ binding sites and clues for clinical relevance. Virchows Arch 427:483–486
7. Faller G, Steininger H, Kränzlein H, Maul H, Kirchner T (1996) Antigastric autoantibodies in *H. pylori* gastritis. Correlation with histologic and serologic parameters. Gut 39 [Suppl 2]:A55
8. Faller G, Steininger H, Kränzlein J, Maul H, Kerkau T, Hensen J, Hahn EG, Kirchner T (1997) Antigastric autoantibodies in *Helicobacter pylori* infection: implications for histological and clinical parameters of gastritis. Gut 41:619–632
9. Gavrieli Y, Sherman Y, Ben-Sasson S (1992) Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol 119:493–501
10. Glass GBJ, Pitchumoni CS (1975) Atrophic gastritis. Structural and ultrastructural alterations, exfoliative cytology and enzyme cytochemistry and histochemistry, proliferation kinetics, immunological derangements and other causes, and clinical associations and sequelae. Hum Pathol 6:219–250
11. Hall PA, Coates PJ, Ansari B, Hopwood D (1994) Regulation of cell number in the mammalian gastrointestinal tract: the importance of apoptosis. J Cell Sci 107:3569–3577
12. Karnes WJ, Samloff IM, Siurala M, Kekki M, Walsh JH (1991) Positive serum antibody and negative tissue staining for *Helicobacter pylori* in subjects with atrophic body gastritis. J Gastroenterol 101:167–174
13. Kasagi N, Gomyo Y, Shirai H, Tsujitani S, Ito H (1994) Apoptotic cell death in human gastric carcinoma: analysis by terminal deoxynucleotidyl tranferase-mediated dUTP-biotin nick end labeling. Jpn J Cancer Res 85:939–945
14. Kato I, Tominaga S, Ito Y, Kobayashi S, Yoshii Y, Matsuura A, Kameya A, Kano T (1992) Atrophic gastritis and stomach cancer risk: cross-sectional analysis. Jpn J Cancer Res 83:1041–1046
15. Kirchner T, Steininger H, Faller G (1997) Immunopathology of Helicobacter pylori gastritis. Digestion 58 [Suppl 1]:14–16
16. Kotani T, Aratake Y, Hirai K, Fukazawa Y, Sato H, Ohtaki S (1995) Apoptosis in thyroid tissue from patients with Hashimoto's thyreoiditis. Autoimmunity 20:231–236
17. Kuipers EJ, Pérez-Pérez GI, Meuwissen SGM, Blaser MJ (1995) *Helicobacter pylori* and atrophic gastritis: importance of cagA status. J Natl Cancer Inst 87:1777–1780
18. Kuipers EJ, Uyterlinde AM, Pena AS, Roosendaal R, Pals G, Nelis GF, Festen HPM, Meuwissen SGM (1995) Long-term sequelae of *Helicobacter pylori* gastritis. Lancet 345:1525–1528
19. Majno G, Joris I (1995) Apoptosis, oncosis, and necrosis. An overview of cell death. Am J Pathol 146:3–15
20. Marshall BJ (1994) Epidemiology of *H. pylori* in Western countries. In: Hunt RH, Tytgat GNJ (eds) *Helicobacter pylori* – basic mechanisms to clinical cure. Kluwer, Dordrecht, pp 75–84
21. Moss SF, Calam J, Agarwal B, Wang S, Holt PR (1996) Induction of gastric epithelial apoptosis by *Helicobacter pylori*. Gut 38:498–501
22. Negrini R, Lisato L, Zanella I, Cavazzini L, Gullini S, Villanacci V, Poiesi C, Albertini A, Ghielmi S (1991) *Helicobacter pylori* infection induces antibodies cross-reacting with human gastric mucosa. Gastroenterology 101:437–445
23. Negrini R, Savio A, Poiesi C, Appelmelk BJ, Buffoli F, Paterlini A, Cesare P, Graffeo M, Vaira D, Franzin G (1996) Antigenic mimicry between *Helicobacter pylori* and gastric mucosa in the pathogenesis of body atrophic gastritis. Gastroenterology 111:655–665
24. Sipponen P, Kekki M, Haapakoski J, Imahäki T, Siurala M (1985) Gastric cancer risk in chronic atrophic gastritis: statistical calculations of cross-sectional data. Int J Cancer 35: 173–177
25. Siurala M, Sipponen P, Kekki M (1985) Chronic gastritis: dynamic and clinical aspects. Scand J Gastroenterol 20 [Suppl 109]:69–76
26. Strickland RG, Mackay R (1973) A reappraisal of the nature and significance of chronic atrophic gastritis. Dig Dis 18: 426–440
27. Stolte M, Baumann K, Bethke B, Ritter M, Lauer E, Eidt H (1992) Active autoimmune gastritis without total atrophy of the glands. Z Gastroenterol 30:729–735
28. Tsuji S, Kawano S, Takei Y, Tsuji M, Kobayashi I, Nagano K, Fusamoto H, Kamada T (1995) Ammonia induces gastric cell apoptosis: possible implication to *Helicobacter*-related gastric mucosal atrophy. Gastroenterology 108:A244