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ROLE OF APOPTOSIS IN GASTRIC EPITHELIAL TURNOVER

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Abstract

Gastric epithelial turnover is a dynamic process. It is characterized by continous cell proliferation, which is counterbalanced by cell loss. The biological principle that mediates the homeostasis of epithelium is programmed cell death, or apoptosis. Currently, several subtypes of apoptosis are distinguished, which are mediated by different mechanisms. Various subtypes of apoptosis also occur in the gastric epithelium under various conditions. In the normal stomach, apoptosis due to cell isolation (*anoikis*) mediates the physiological epithelial turnover. Albeit rarely seen in routine histology, approximately 2% of epithelial cells in the normal stomach are apoptotic. In *Helicobacter pylori*-induced gastritis, apoptosis and epithelial proliferation are moderately increased, with approximately 8% apoptotic epithelial cells. In gastritis, factors such as CD95 ligand or tumor necrosis factor (TNF) alpha act as death factors. They bind to specific receptors, CD95 and TNF-R, which are induced either by other cytokines, such as interferon gamma, or by *Helicobacter pylori* itself. In addition to CD95, *H. pylori can* also induce upregulation of CD95 ligand expression. Taken together, the upregulated expression of CD95, and the presence of CD95L in the close proximity to apoptotic gastric epithelial cells suggest a functional role of the CD95-CD95L system in the induction of apoptosis in *H. pylori*-gastritis. The role of other pathways to apoptosis is currently under study. Apart from being a biological phenomenon, apoptosis in the stomach may also have direct clinical consequences. An extreme example is given in gastric graft-versus-host disease when epithelial denudement occurs.

Introduction

Gastric epithelial turnover is a dynamic process. It is characterized by continous cell proliferation, which is counterbalanced by continous cell loss. The biological phenomenon that mediates the homeostasis of epithelium is programmed cell death, or apoptosis (Kerr et al., 1972; Wyllie et al., 1980; Thompson, 1995; Raff, 1998). This article will focus on the role of apoptosis in the normal gastric mucosa and in chronic gastritis.

A view on epithelial turnover warrants consideration of gastric histology, which differs in the four anatomical subsites of the stomach. These four zones, i.e. cardia, fundus, corpus, and antrum (as defined for the TNM classification system) (UICC, 1997) are lined by two basically different types of mucosa. One type is present in the upper and middle third of the stomach, i.e. fundus and corpus (UICC, 1997). This fundus-type or oxyntic mucosa is characterized by rather straight, composite glands lined by mucous neck cells, chief (or zymogenic) cells, parietal cells, and neuroendocrine cells (Owen, 1997). A second type of mucosa is present in the distal third of the stomach, i.e. antrum and pylorus, and also at the cardia (Owen, 1997). This antrum-type mucosa is characterized by more branched, predominantly mucoid glands with few neuroendocrine cells and sparse parietal cells (Owen, 1997). Apart from the different glands, fundus-type and antrum-type mucosa differ also with respect to the luminal openings of their glands, the foveolae or pits. In the antrum, foveolae comprise approximately one-half of the mucosal thickness (Owen, 1997). In the corpus and fundus, the foveolar length occupies approximately one-quarter of mucosal thickness (Owen, 1997). In any localization, the gastric pits are lined by cylindric mucinous epithelium (Owen, 1997).

Gastric epithelial proliferation

Gastric mucosa differs from other digestive tract mucosae with respect to its proliferative zone. In any subsite of the stomach, the proliferating stem cells are localized in the neck of gastric glands, and epithelial migration occurs bidirectionally. One part of proliferated epithelia migrates within few days upwards to the luminal surface, and cells differentiate into columnar mucous-secreting epithelia which line the foveolae ("pits") and the surface (Owen, 1997). Other proliferated epithelia migrate downwards in the gastric glands. In the antrum, they differentiate within few days to mucous-secreting cells forming the pyloric glands. In the fundus and corpus, they differentiate within few weeks to chief cells, parietal cells, and neuroendocrine cells (Owen, 1997). By means of electron microscopy, their suspected precursor cells can be identified (Karam and Leblond, 1992; 1993a-e).

Proliferation kinetics of the gastric antral and fundus-type mucosa differ significantly. Most of the early studies have concentrated on proliferation of the foveloar epithelium (Lipkin et al., 1963; Hansen et al., 1979). By means of bromodeoxyuridine (BrdU) labelling, 4.8% of epithelial cells in the antrum, but 2.8% in the gastric body epithelium were found to be in the in DNA synthesis (S-) phase of the cell cycle (Patel et al., 1993). The mean duration of S-phase of gastric antral cells was 7.7 hours, and of gastric body epithelium was 10.8 hours (Patel et al., 1993). The median lengths of the foveolae were 188 cells in the gastric antrum, and 137 cells in the gastric body (Patel et al., 1993). Correspondingly, the median peak of BrdU-labelling positions were cell 61 in the antrum, and cell 26 in the gastric body as measured from the crypt orifice (Patel et al., 1993). Epithelial proliferation is moderatly increased in gastric remnants after partial gastric resection (Lynch et al., 1995).

Gastric epithelial renewal is influenced by various physiologic stimuli. In the gastric fundus and corpus, gastrin effectively stimulates proliferation of epithelial cells. In the antrum, however, epithelial proliferation is hardly influenced by gastrin. Clinical evidence for this divergent effects is given in chronic hypergastrinemia, i.e. gastrinoma (or Zollinger-Ellison-) syndrome.

Apoptosis in the stomach: general aspects

The diversity of cell death was recognized more than a century ago by Rudolf Virchow. The traditional term necrosis, however, was redefined only in 1972, when the new term apoptosis was coined (Kerr et al., 1972). Conceptually, apoptosis is an active and regulated process by which cells self-destruct. In contrast, necrosis is a passive cell death following irreversible injury. Morphologically, necrosis and apoptosis are two distinctive types of cell death (Wyllie et al., 1980; Majno and Joris, 1995; Cummings et al., 1997). Beside them, there appear to be at least two further types of cell death which have different morphological features (Schweichel and Merker, 1973).

Necrosis, in modern terms, is morphologically characterized by overall cell and organelle swelling, with subsequent early loss of membrane integrity which is followed by cell and organelle lysis (Majno and Joris, 1995). Due to the release of cellular constituents, necrosis is accompanied in vivo by an inflammatory response. Biochemically, necrosis is characterized by random degradation of nuclear DNA.

Apoptosis, in contrast, is morphologically characterized by chromatin condensation, as well as by cytoplasmic condensation (Wyllie et al., 1980; Majno and Joris, 1995; Cummings et al., 1997). An important and characteristic feature is the presence of intact organelles, cell constituents are not released. As a consequence, no inflammatory response is apparent around apoptotic cells. Fragmentation of the nucleus and convulation of cytoplasmic membranes is followed by overall cell shrinkage and the formation of so-called apoptotic cell bodies. These bodies are phagocytosed by neighbouring cells. Biochemically, the hallmark of apoptotic cell death is nonrandom degradation of DNA (see below).

Regulation of apoptosis is complex and occurs from both outside and inside the cell (Raff, 1998). Extracellular signals can either suppress or activate apoptosis. Apoptosis-suppressing ("negative signalling") molecules are survival factors, e.g. growth factors. Apoptosis-activating ("positive signalling") factors are death-inducing molecules, e.g. TGF-β or related peptides (inhibins, acticins, Müllerian inhibiting factor), CD95 ligand, or tumor necrosis factor alpha (TNF α) and related molecules. These signals act via binding to specific receptors on their target cell surface.

One mechanism of inducing apoptosis is activation of the CD95 receptor and ligand system (Krammer, 1999). The CD95 molecule (synonyms: APO-1, Fas) is a type I transmembrane receptor which belongs to the nerve growth factor/tumor necrosis factor receptor superfamily (Krammer, 1999; Weiss et al., 1997). CD95 is constitutively expressed in a wide range of normal human tissues (Leithäuser et al., 1993). Moreover, expression of CD95 can be induced in various conditions (Leithäuser et al., 1993). CD95 ligand (CD95L) is a transmembrane protein which belongs to the TNF family (Krammer, 1999). CD95 ligand is expressed in various human cells and tissues, such as activated T lymphocytes, lung, liver or kidney (Suda et al., 1993; 1994; Galle et al., 1995). Binding of CD95L to the extracellular

domain of CD95 induces trimerization of the receptor (Krammer, 1999). Then, the intracellular domain of CD95 recruits via an adaptor FADD (Fas associated death domain) the cytoplasmic caspase-8 (FLICE/ MACH/Mch5; see below) (Nagata, 1997).

Similarly, binding of tumor necrosis factor (TNF) to the extracellular domain of the TNF receptor (CD120a,b) induces receptor trimerization, allowing the intracellular domain of CD120 to recruit the adaptor proteins TRADD (TNF receptor associated death domain), TRAF2 (TNF receptor associated factor), and RIP (receptor interacting protein) (Ashkenazi and Dixit, 1998). This complex then activates further intracellular cascades.

Alternatively, apoptosis can also be initiated by other pathways (Raff, 1998). Some cells, such as killer lymphocytes, secrete various proteins onto the surface of their targets cells. One of the proteins, perforin, can assemble into transmembrane channels that allow other proteins to enter into the cell. Granzyme B is a protease that cleaves and activates intracellular proteases (see below) (Atkinson and Bleakley, 1995).

Upon initiation by either mechanism, specific intracellular proteases become activated which are members of a family of cysteine aspartic acid-specific enzymes, termed caspases (Alnemri et al., 1996; Nicholson and Thornberry, 1997; Rosen, 1996; Vaux and Strasser, 1996). Caspase-1 is a human homologue of the nematode *Caenorhabditis elegans* cell death gene *ced-3*, and is identical with the human interleukin-1 converting enzyme (ICE) (Yuan et al., 1993). Activated ICE/CED-3 proteases can cleave various substrates, e.g. lamins, actin, poly-ADP ribose polymerase (Nagata, 1997). They are capable to bring about the systematic destruction of cells. Until now, the caspase family consists of ten different members (Krammer, 1999).

A major class of intracellular regulators of apoptosis is the Bcl-2 protein family (Adams and Cory, 1998; Raff, 1998). The human *bcl-2* gene is the homologue to the worm *C. elegans* cell death gene *ced-9* (Raff, 1998). It encodes for a 26 kD protein. The Bcl-2 family encompasses several proteins, many of which can physically interact to form homo- and heterodimers with each other. The biological effects are quite different. Bcl-2, Bcl-x_L, Mcl-1, and Bfl-1 suppress apoptosis, by inhibiting adapters needed for activation of the caspases that dismantle the cell (Adams and Cory, 1998). Other family members, e.g. Bax, Bak, Bad and Bid, instead promote apoptosis, apparently through mechanisms that include displacing the adapters from the pro-survival proteins (Adams and Cory, 1988). Thus, the balance between these competing regulatory molecules determines cell fate. Beside the Bcl-2 protein family, there is at least one further family of intracellular regulators, termed the IAP (inhibitors of apoptosis) proteins (LaCasse et al., 1998).

A biochemical hallmark of apoptotic cell death is nonrandom degradation of DNA. This occurs in two-steps, mediated by activated caspases (see above) which cleave a protein that normally keeps a DNA degrading enzyme in an inactive form (Raff, 1988). In a first step, larger fragments of approximately 300 and 50 kb length are cleaved, probably through a Mg^{2+}-dependent DNase, topoisomerase II (Oberhammer et al., 1993). In a second step, smaller oligonucleosomal fragments results through the action of Ca^{2+}-dependent endonuclease.

DNA fragmentation is also a key to morphological studies of apoptosis. Specific labelling of nuclear DNA fragments allows visualization of apoptotic cells in tissue sections. Many studies have applied the TdT (terminal deoxynucleotidyl transferase)-mediated dUTP nick end labelling (TUNEL) method for this purpose (Gavrieli et al., 1992).

Apoptosis in the normal gastric mucosa

Physiological epithelial cell loss in the stomach is mediated by apoptosis, thus it is an active process (Hall et al., 1994; Stachura et al., 1993; Sterle and Pipan, 1994). Committed cells detach from neighbouring epithelia, as well as from their extracellular matrix. This disruption of cell-cell- or cell-matrix-interaction may result in a loss of external suppression of apoptosis (Frisch and Francis, 1994). Eventually, apoptotic cells are shed into the lumen. As an alternative term for this subtype of active cell death due to cell isolation, *anoikis* was proposed (derived from greek, meaning homelessness) (Frisch and Francis, 1994).

By means of the TUNEL method, approximately 1-3% of gastric epithelial cells in the antrum or corpus/fundus are apoptotic (Figure 1) (Imatani et al., 1996; Ishida et al., 1996; Moss et al., 1996; Rudi et al., 1998a; Steininger et al., 1998).

Apoptotic bodies are only rarely identified by light microscopy in the normal gastric mucosa. This virtual rarity may be due to confounding with intraepithelial lymphocytes, and to the disposition of membrane-bound apoptotic bodies into the glandular lumen where they present as eosinophilic granular debris (Washington et al., 1987). When apoptotic bodies are present, they are either engulfed by adjacent epithelial cells, or localized in the immediate subepithelial connective tissue (Hall et al., 1994). Of note, apoptotic bodies are not randomly distributed but are found towards the upper and lower end of the gastric glands (Hall et al., 1994; Imatani et al., 1996; Ishida et al., 1996).

Beside senescent apoptosis, it is assumed that there is another subtype of apoptosis in the normal gastric mucosa, which becomes relevant in the elimination of cells with DNA damage and genomic mutations (Shirin and Moss, 1998). Such an altruistic phenomenon of self-desctruction was suggested to occur in the intestinal crypts of irradiated rodents (Potten, 1992).

An early screening study suggested the absence of CD95 (APO-1/Fas) expression in any epithelial cells of the normal stomach (Leithäuser et al., 1993). Later studies, however, revealed that CD95 is constitutively expressed in few gastric epithelia (Houghton et al., 1999; Rudi et al., 1998a). Albeit the number of foveolar epithelia which express CD95 is small, it correlates with approximately half the number of apoptotic cells (Rudi et al., 1998a). Thus, the CD95 pathway to apoptosis might also be involved in the physiological turnover of gastric epithelial cells.

Stem cell homeostasis in the stomach may be mediated by apoptosis, such as it is in the mammalian small intestine and colon, but evidence for this assumption is limited (Potten, 1998). Only few TUNEL+ cells are present in the neck zone of gastric glands where proliferating cells (Ki67+) prevail (Ishida et al., 1996).

Intracellular regulatory proteins of the Bcl-2 family are expressed in different patterns in the normal gastric epithelium. By means of immunohistochemistry, Bcl-2 protein was found to be expressed in all epithelial cell types of the normal stomach, with a tendency to be more intensively expressed in the neck region (Krajeweski et al., 1995). In another study, however, immunostaining was found to be limited to a few cells in the mucous neck region (Cho and Kim, 1998). The *mcl-1* gene product, a 37-kd protein, is expressed in the gastric foveolar epithelium, but not in glandular epithelia (Krajewski et al., 1995). When compared by means of immunoblotting, Bcl-2 protein is more abundant than Mcl-1 protein in the normal stomach (Krajewski et al., 1995). The different patterns of Bcl-2 and Mcl-1 immunostaining suggests that these proteins fulfill different roles in the

physiology of cell death regulation (Krajewski et al., 1995). High amounts of Bax protein are present in the gastric glands, as revealed by immunohisto-chemistry and Western blot analysis (Penault-Llorca et al., 1998). The lack of a correlation between Bcl-2, Bax, and apoptotic features suggests that Bax alone is not sufficient to trigger cell death, but may modulate the role of other regulators of apoptosis (Penault-Llorca et al., 1998). Bfl-1 is apparently not synthesized in gastric foveolar epithelial cells, as revealed by negative findings by means of in situ hybridization (Jung et al., 1998).

Caspase-3, a protein product of CPP32 gene which is a human homologue of the worm cell death gene *ced-3*, is present in the cytosol of human gastric epithelial l cells (Krajewska et al., 1997).

Apoptosis in chronic gastritis

Inflammatory gastric diseases are commonly associated with increased epithelial proliferation. Several studies have shown this phenomenon in *H. pylori*-induced chronic gastritis, by means of immunohistochemical detection of proliferating cells (Ki67; PCNA), by silver staining of nucleolar organizer regions (AgNOR), and other techniques (Brenes et al., 1993; Cahill et al., 1995; Fraser et al., 1994; Harvard et al., 1996; Lynch et al., 1995; Megraud et al., 1992; Moss et al., 1996; Murakami et al., 1995; Tsuji et al., 1992). In addition, epithelial hyperproliferation also occurs independently of *H. pylori* infection (Fraser et al., 1994; Chow et al., 1995; Nardone et al., 1999). Of note, chronic gastritis is usually not associated with mucosal thickening, indicating that epithelial hyperproliferation is apparently balanced with cell losses. The major mechanism of epithelial cell loss in type B (B=bacterial, *H. pylori*-induced) and type C (C=chemically-induced) gastritis is apoptosis, while the pathogenesis of type A (A=autoimmune) gastritis is less clear.

Chronic gastritis, type A

Autoimmune gastritis is characterized by diffuse lympho-plasmacellular infiltration in the fundus-type mucosa, associated with progressive loss of parietal cells and chief cells (Eidt et al., 1996). At long-term (< 20 years), mucosal atrophy with antrum-like gland remnants results. Although autoantibodies against parietal cell H^+/K^+-ATPase or intrinsic factor (produced by parietal cells) are present in about 90% of patients, it is considered unlikely that these autoantibodies are pathogenic (Toe et al., 1997). In contrast to some other autoimmune diseases, autoimmune gastritis usually lacks lympho-epithelial lesions. Thus, the pathogenesis of parietal cell and chief cell loss in type A chronic gastritis is poorly understood. Apoptosis mediated by the CD95 (APO-1/Fas) pathway is a possible, but yet unproven mechanism (Nishio et al., 1996).

Chronic gastritis, type B

Gastritis induced by *Helicobacter pylori* is the most common gastric disease. It is characterized by diffuse or superficial infiltration of the lamina propria by mononuclear cells and neutrophils, in the presence of bacterial colonization but not invasion (Dixon et al., 1996).

Mild increase of gastric epithelial proliferation is a common feature in *H. pylori*-induced gastritis. By means of immunohistochemistry, the percentage of bromodeoxyuridine (BrdU)-labelled foveolar cells in the S-phase of the cell cycle increased from 3.3% (mean value; SD 1.5-5.6) in H.p.-negative patients to 5.4% (mean value; SD 1.0-11.7) in *H.pylori*-positive patients (Anti et al., 1998). There are several possible mechanisms to explain this increased proliferation, which may act in concert. First, it may caused by *H. pylori* urease

activity producing ammonia (Tsuji et al., 1993; Matsui et al., 1997). Second, *H. pylori*-associated hypergastrinemia may play a role. Third, *H. pylori*-induced inflammatory response may determine epithelial cell kinetics (Anti et al., 1998).

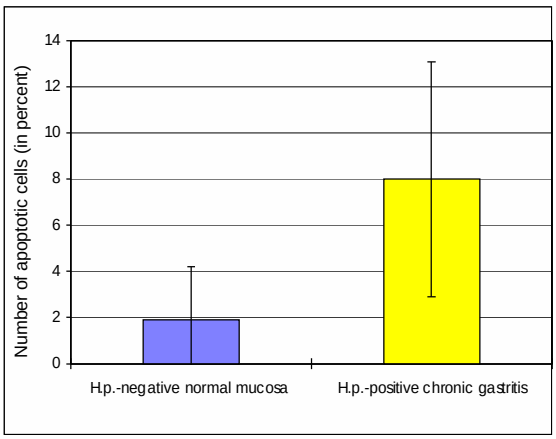

Fig. 1 Gastric epithelial apoptosis in the normal antrum (H.p.-negative), and in chronic antral gastritis (H.p.-positive). Biopsies were assessed after TUNEL staining, and at least 300 cells were counted in each histologic section. The number of positive cells per 100 epithelial cells is expressed as percentage (data from: Rudi et al., 1998).

Correspondingly, a mild to moderate increase in the number of apoptotic cells is rather consistently found in type B gastritis (Figures 1 and 2). Between 3% and 16% apoptotic epithelial cells were counted (Anti et al., 1998; Atallah et al., 1996; Hahm et al., 1997; Jones et al., 1997; Mannick et al., 1996; Moss et al., 1996; Rudi et al., 1998a; Steininger et al., 1998). This range may be explained by counting of various cells (foveolar vs. glandular epithelium, or both), and by the different virulence factors of *H. pylori* strains (Rudi et al., 1998b). However, no association was found between the patient´s CagA status and apoptosis (Peek et al., 1997; Zhu et al., 1998). Interestingly, in one study of those patients infected with *cagA*+ *vacA* s1a strains of *H. pylori* apoptosis was observed to be less increased than epithelial proliferation (Peek et al., 1997). This dissociation of proliferation and apoptosis was suggested as a possible explanation for the heightened risk of gastric carcinoma that is associated with infection by CagA+ and VacA+ strains of *H. pylori* (Blaser et al., 1995; Rudi et al., 1997).

Decrease of apoptosis was consistently observed after eradication of *H. pylori* (Moss et al., 1996; Mannick et al., 1996; Hahm et al., 1997; Jones et al., 1997; Nardone et al., 1999). Thus, the increase of apoptosis in active *H. pylori*-infection is a reversible phenomenon.

In a subgroup of patients with *H. pylori*-gastritis extending from the antrum into the corpus, antigastric antibodies are present which bind to the canaliculi within parietal cells. The presence of these antibodies correlates with the severity of gastritis in the body and with glandular atrophy. In those patients, a small increase (3%, vs. 1%; $p < 0.05$) of apoptotic cells (TUNEL+) was found in the corpus foveolar and glandular epithelium (Steininger et al., 1998).

Common view suggests that apoptosis is a direct effect of *H. pylori* infection, and is not an effect of the inflammatory infiltrate which characterizes *H. pylori*-gastritis (Moss et al., 1996). This view was supported by experimental findings in rats where lipopolysaccharide prepared from a well characterized *H. pylori* strain (MTCC 11637) induced apoptosis (Piotrowski et al., 1996).

Various mechanisms of induction of apoptosis in chronic gastritis caused by *Helicobacter pylori* are possible. The common mechanism appears to involve the CD95 (APO-1/Fas) receptor and ligand system. Evidence for this is provided by studies in vivo (Rudi et al., 1998a;

Houghton et al., 1999) and in vitro (see below).

In gastritis, the number of epithelial cells with the CD95 receptor molecule is substantially increased, both in the antral surface epithelium (30%, vs. 0,8% in non-inflamed stomach; $p < 0.003$), and in the pyloric glands (76%, vs. 53% in the normal antrum; $p < 0.018$) (Figure 3) (Rudi et al, 1998a). This upregulation in CD95 expression might be expected to result from the secretion of pro-inflammatory cytokines such as interferon-γ or TNF-α by infiltrating leucocytes, but experimental data argue against a major role of these cytokines (see below). Alternatively *H. pylori* appears itself to induce CD95 receptor expression. Evidence for this is provided by in vitro studies (see below).

CD95 ligand (CD95L) is expressed by the infiltrating lymphocytes in the gastric mucosa, and interestingly also by the epithelial cells themselves (Houghton et al., 1999; Rudi, et al., 1998a). CD95L might be expressed in a membrane bound form on epithelial cells by contact with *H. pylori*. Epithelial CD95L may mediate apoptosis by "fratricide" interacting with CD95 on neighboring epithelial cells, or through suicide of the CD95-CD95L expressing cells itself. The upregulated expression of CD95, and the presence of CD95L in the close proximity to apoptotic (TUNEL+) gastric epithelial cells suggest a functional role of the CD95-CD95L system in the induction of apoptosis in *H. pylori*-gastritis (Rudi et al., 1998a).

No significant upregulation of Bcl-2 protein was found in *H. pylori*-gastritis of non-*H. pylori*-gastritis (Chen et al., 1997; El Kaissouni et al., 1998). However, expression of Bak protein was reported to be increased in *H. pylori*-gastritis (Chen et al., 1997)

Apoptosis in type B gastritis may have clinical implications. Some have hypothesized that gastroduodenal ulcer lesions result from apoptotic cell losses which are not counterbalanced by epithelial hyperproliferation (Houghton et al., 1999). Indeed, an overall increase of apoptosis was observed, by means of DNA flow cytometry of epithelial cells expressing epidermal growth factor receptor, in patients with *H. pylori*-infection and gastroduodenal ulcer disease (Kohda et al., 1999). Parallel TUNEL staining indicated an increase of apoptosis in pyloric gland epithelia (Kohda et al., 1999). Among the possible virulence factors individual *H. pylori* strains, a relationship was observed for urease activity, but not for vacuolating toxin activity, and not for the number of colony forming units of bacteria (Kohda et al., 1999). Moreover, others have suggested that increased apoptosis, compared with epithelial proliferation, may explain the outcome of *H. pylori*-induced gastritis with mucosal atrophy.

Chronic Gastritis, Type C

Gastritis induced by chemical agents, e.g. bile reflux, drugs, alcohol, is frequently characterized by mucosal edema and erosions in the presence of a rather scarce leucocyte infiltration in the lamina propria. Apoptosis is also a mechanism of type C gastritis. In patients with rheumatoid arthritis treated with non-steroidal anti-inflammatory drugs (NSAID), apoptosis is significantly increased (Zhu et al., 1998). Surprisingly, in those patients with concurrent *H. pylori*-gastritis the increase of apoptosis is partly reversed when NSAID are taken (Zhu et al., 1998). In experimental alcohol-induced gastropathy, as early as 30 minutes after ingestion of alcohol mucosal lesions are present in the stomach which are accompanied by a 9.5-fold increase in apoptosis, and a 2.5-fold increase in TNF-alpha. These effects could be reduced by antiulcer agents, such as ebrotidine, omeprazole, and sucralfate (Piotrowski et al., 1997). Similar observations were reported for gastric injury induced by indomethacin (Slomiany et al., 1997).

Gastric Graft-versus-host Disease

Apoptosis is the hallmark of graft-versus-host disease (GvHD) in the stomach, which occurs rather exclusively after allogeneic bone marrow transplantation (Washington et al., 1997). Beside the classic target organs of GvHD (i.e. skin, liver, intestine, bone marrow) gastric involvement is apparently more common than previously appreciated (Snover et al., 1985; Washington et al., 1997). Usually numerous large apoptotic bodies are present which tend to be located in the neck portion of the gland, while sometimes they are small and inconspicious (Washington et al., 1997). In extreme cases epithelial denudement occurs in the stomach, while the lamina propria appears rather intact (Thorning and Howard, 1986). As there is only a sparse lamina propria infiltrate, if any, apoptosis in GvHD is assumed to be induced be cytokine dysbalance (Ferrara et al., 1996; Thorning and Howard, 1986; Washington et al., 1997).

Intestinal metaplasia

Mucosal injury of various causes may eventually result in gastric mucosal atrophy, as defined by the presence of metaplastic glands with intestinal-type epithelium (Dixon et al., 1996; Genta, 1997). Intestinal metaplasia is considered to be a precancerous condition, while epithelial dysplasia is a precancerous lesion. Therefore, some studies have attempted to characterize gastric epithelial turnover in intestinal metaplasia. Overall cell turnover was found to be slightly increased (Imatani et al., 1996; Ishida et al., 1996). The proliferation zone of metaplastic intestinal-type glands is localized at the base, such as in the intestine but different from normal gastric glands (Imatani et al., 1996; Ishida et al., 1996). Apoptotic cells (TUNEL+) are found both at the base and at the surface (Imatani et al., 1996; Ishida et al., 1996). Interestingly, apoptosis was somewhat more frequent in glands with incomplete than complete intestinal metaplasia, presumably indicating elimination of more DNA-damaged cells (Ishida et al., 1996). This is consistent with the finding that Bcl-2 protein, which inhibits apoptosis, is expressed in most cases (91%) of incomplete intestinal metaplasia, but only in half of cases (56%) with complete intestinal metaplasia (Saegusa et al., 1995).

Mechanisms of apoptosis in gastric epithelial cell lines

Several experimental studies were performed in gastric epithelial cell lines, all of which are derived from human gastric adenocarcinomas. Many of them have investigated the effects of chemotherapy (e. g. 5-fluorouracil, cisplatin) or other drugs (e.g. paclitaxel, green tea catechin extracts) to inhibit cancer cell growth, thereby looking at cell death as the end point of study (Chang et al., 1996; Inada et al., 1997; Ikeguchi et al., 1997; Hibasami et al., 1998; Vollmers et al., 1995). Beside these kind of studies, some other have concentrated on the cellular mechanisms of apoptosis in the stomach.

The capacity of transforming growth factor beta (TGF-β) to induce apoptosis was reproduced in a dozen gastric cancer cell lines. Binding of TGF-β to TGF receptors I or II on the surface of gastric cancer cells resulted consistently in activation of the apoptosis cascade (Yamamoto et al., 1996). In another study, this effect was shown to be mediated by a CPP32-like protease (Ohta et al., 1997).

The biological effects of tumor necrosis factor (TNF) α and its soluble TNF receptors were investigated in gastric cell lines MKN45 and Kato-III. Supernatants from cultures of gastric mucosal biopsies, as well as from gastric epithelial cell lines, were analyzed with regard to the concentration of TNF-α and soluble TNF receptors (sCD120a; sCD120b). Soluble

TNF receptors, both sTNF-R type I (55kDA) and sTNF-R type II (75kDa), were found to be actively produced in *H. pylori* infected gastric mucosa. The sTNF-R appeared to counteract the effects of TNF in vitro to induce apoptosis in gastric epithelial cells in *H. pylori* infection (Shibata et al., 1999).

An alternative pathway to induce apoptosis was suggested in a recent study. In a gastric cancer cell line (Kato-III), apoptosis was induced by cross-linking IgM antibodies to surface major histocompatibility complex (MHC) class II molecules in the presence of *H. pylori* (Fan et al., 1998). This effect was partially inhibited by blocking MHC class II molecules with antibody. These data indicate a possible role of MHC class II molecules in the induction of apoptosis in *H. pylori*-gastritis (Fan et al., 1998).

Mediation of apoptosis induced by anti-CD95 (APO-1/Fas) antibody was shown in six human gastric carcinoma cell lines characterized by either deleted, or mutated, or wild-type p53 gene. Apoptosis occured in a delayed fashion. At 72 h, between 35 to 60% of cells with wild-type p53, but only 20% of cells with mutated or deleted p53 gene were apoptotic (Hayashi et al., 1997).

The possible role of *Helicobacter pylori* itself to induce apoptosis was investiganted in few studies. Cocultivation of gastric cancer cell line HM02 (established from a mucus-producing human gastric carcinoma) with *H. pylori* strains was associated with a time- and dose-dependent reduction of increase in cultured cell counts (Wagner et al., 1997). This effect was shown to be due to direct induction of apoptosis and inhibition of DNA synthesis, and indirectly by sensitization of cancer cells for apoptosis induced by proinflammatory stimuli (Wagner et al., 1997). Similiarly, cocultivation of other gastric cancer cell lines (AGS, Kato-III, Hs746T) with *H. pylori* supernatant resulted also in a rapid increase in apoptotic cells (Chen et al., 1997; Rudi et al., 1998a). Conflicting results were reported about the possible role of virulence factors. One study observed no significant differences between *cagA*-positive or *cagA*-negative strains of *H. pylori* (Wagner et al., 1997). Another study found a significant difference in the rate of apoptotic cells when the cells were incubated with a cytotoxic *H. pylori* strain (i.e. a *vacA* s1 and *cagA* positive strain) compared with a non-cytotoxic strain (Rudi et al., 1998a).

Concering the mechanisms of *H. pylori*-induced apoptosis, the expression of CD95 was studied in various gastric epithelial cells lines (AGS, Kato-III, Hs 746T). Incubation of cells with sublethal doses of a cytotoxic *H. pylori* supernatant resulted in increased numbers of CD95+ cells, from a basal expression in 7% of cells up to 50% of AGS cells within 48 hours. Interestingly, this effect of incubation with *H. pylori* supernatants was more prounounced than with interferon-γ and TNF-α (Rudi et al., 1998a; Wagner et al., 1977). Similiar observations were also reached with non-gastric cancer cell lines (HeLa and HepG2 cells) (Rudi et al., 1998a). The mechanism of this effect is so far unknown.

Another effect of *H. pylori* was observed in studies on the expression of CD95 ligand in gastric epithelial cell lines (AGS, Kato-III; Hs 746 T). In untreated cells, CD95L mRNA was constitutively expressed. After incubation with cytotoxic *H. pylori* supernatant, levels of CD95L mRNA were increased (Rudi et al., 1998a). Further support for an active epithelial production of CD95L was given by another experiment. After pretreatment with *H. pylori* supernatant, gastric epithelial cell (AGS) supernatant increased apoptosis in a CD95L-sensitive lymphoma line (Jurkat). This implies a functional role of CD95L (Rudi et al., 1998a).

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Legends to the other figures

Fig.2 Apoptotic bodies (*) at the surface of foveolar glands in active *H. pylori*-gastritis in the antrum (hemalaun-eosin, original slide magnification x158).

Fig. 3 Expression of CD95 receptor in glandular and foveolar epithelial cells in chronic *H. pylori*-gastritis (immunohistochemistry; x400).