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***Medical Hypothesis***

# A concept on the role of *Helicobacter pylori* infection in autoimmune pancreatitis

**J. Kountouras*, C. Zavos, D. Chatzopoulos**

*Department of Medicine, Second Medical Clinic, Aristotle University of Thessaloniki, Ippokration Hospital, Thessaloniki, Greece*

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## Abstract

Autoimmune pancreatitis, an inflammatory process of the pancreas due to an autoimmune mechanism establishing etiology of chronic pancreatitis, is characterized by the presence of autoantibodies, hypergammaglobulinemia, pancreatic enlargement, pancreatic duct strictures, and pathologic features of fibrotic changes with intense, mainly lymphocytic infiltrations, which may contribute to tissue destruction probably by apoptosis. In almost 60% of the cases, this type of pancreatitis coexists with other autoimmune diseases such as Sjögren's syndrome, sclerosing extrahepatic cholangitis, primary biliary cirrhosis, autoimmune hepatitis, or other extrapancreatic disorders, and recently with gastric peptic ulceration. The diversity of extrapancreatic lesions with similar histopathologic findings suggests general involvement of the digestive system in this disease, although the presence of such involvement has not been fully elucidated. Similarly, *Helicobacter pylori (H. pylori)* infection, a well known cause of gastric ulcer, has been associated, via molecular mimicry of host structures by its constituents with the same autoimmune conditions, also characterized by fibrotic changes and/or lymphoplasmacytic inflammations, accompanied by aberrations of T cell apoptosis that contribute to hepatobiliary- or extrahepatic-tissue destruction. Considering that *H. pylori* is involved in the pathogenesis and pathophysiology of these autoimmune disorders, we propose that this organism might trigger autoimmune pancreatitis through induction of autoimmunity and apoptosis.

**Keywords**: autoimmune pancreatitis • *Helicobacter pylori* • molecular mimicry • apoptosis • T cells

* Correspondence to: Jannis KOUNTOURAS, MD, PhD
Associate Professor of Medicine, Gastroenterologist
8 Fanariou St, Byzantio 551 33,Thessaloniki, Macedonia, Greece
Tel.: +30-2310-892238, Fax: +30-2310-992794
E-mail: jannis@med.auth.gr

## Introduction

Since Sarles *et al* [1] observed a case of specific pancreatitis associated with hypergammaglobulinemia that was not associated with alcohol consumption, subsequent reports have described patients with chronic pancreatitis characterized by the presence of autoantibodies, elevated levels of immunoglobulins (IgG4), enlargement of the pancreas (diffuse or focal), pancreatic duct strictures, and pathologic features of fibrotic changes with an intense inflammatory cell (mainly lymphocytic) infiltration [2-6]. These major lymphocytic infiltrations may contribute to tissue destruction probably by apoptosis [7-10]. In approximately 60%, the coexistence of this type of pancreatitis with other autoimmune diseases such as Sjögren's syndrome (SjS), sclerosing extrahepatic cholangitis [interpreted as a variant of primary sclerosing cholangitis (PSC) or as an inflammatory pseudotumor], primary biliary cirrhosis (PBC), autoimmune hepatitis (AIH), or other extrapancreatic disorders, such as retroperitoneal fibrosis, salivary gland swelling, inflammatory bowel disease (IBD), Hashimoto's thyroiditis, and recently gastric peptic ulceration has been reported [4-6,11]. The histopathologic findings in these extrapancreatic lesions are lymphoplasmacytic inflammation and fibrosis, similar to those in the pancreatic tissue, suggesting a common pathogenesis [12-17]. The diversity of extrapancreatic lesions with similar histopathologic findings suggests general involvement of the digestive system in this disease, although the presence of such involvement has not been fully elucidated. In addition, cases without systemic autoimmune diseases have been reported, which has led to the concept of an autoimmune related pancreatitis [2,3], so called "autoimmune pancreatitis" (AIP), proposed by Yoshida [2]. These findings support the hypothesis that an autoimmune mechanism may be involved in the pathogenesis and pathophysiology in a proportion of patients with pancreatitis [17-21], possibly mediated by an overactive apoptosis [7-10,22].

In the same respect, *Helicobacter pylori* infection has been strongly associated with peptic ulceration of the stomach [23] and gastric autoimmunity [24], and patients infected with *H. pylori* have been shown to possess autoantibodies that cross-react with antigens expressed on the gastric mucosa [24]. Moreover, as in the case of AIP, *H. pylori* is associated with autoimmune conditions like SjS (i.e., autoimmune sialadenitis), PBC, PSC, AIH or hepatitis C virus (HCV)-related liver disease which trigger autoimmune sequelae (AIH and HCV-related SjS) [25-35]. These *H. pylori*-related diseases are also characterized by fibrotic changes and/or lymphoplasmacytic inflammations [28,35-39], accompanied by aberrations of T cell apoptosis that contribute to hepatobiliary- or extrahepatic-tissue destruction [35,36,40-46]. Considering that *H. pylori* is involved in the pathogenesis and pathophysiology of the above mentioned autoimmune disorders [27-31,33], we propose that this organism might trigger AIP through induction of autoimmunity and apoptosis [47].

## Definition and terminology of AIP

AIP can be defined as an inflammatory process of the pancreas due to an autoimmune mechanism establishing etiology of chronic pancreatitis [48]. Cases of isolated (primary) AIP without other autoimmune diseases have been reported. Many other terms, such as "primary inflammatory pancreatitis", "primary chronic pancreatitis", "non-alcoholic duct-destructive chronic pancreatitis", "lymphoplasmacytic sclerosing pancreatitis", "pseudotumorous pancreatitis", "granulomatous pancreatitis", "chronic inflammatory sclerosis of the pancreas", "pancreatitis showing the narrowing appearance of the pancreatic duct", and "sclerosing pancreatocholangitis" have also been used in the literature to identify AIP [4-6,49,50]. In addition, some authors defined "autoimmune exocrinopathy", "dry gland syndrome", or "a complex syndrome", as the concurrent involvement of the pancreas, the salivary glands (SjS) and the liver (PBC) [5,49]. However, it is poorly understood whether the pathogenetic mechanism of syndromic (or secondary) AIP with other autoimmune diseases is different from primary AIP. It was thought that there is a possibility of developing systemic autoimmune diseases in patients previously diagnosed as having primary AIP [3,48].

Strictly speaking, the classification of a disease as autoimmune requires a number of clinical/biochemical and experimental criteria not applicable to

human diseases [51]. A number of criteria have been suggested as indicative for an autoimmune pathogenesis [51]: 1) the presence of autoimmune antibodies specific for the disease and/or the presence of non-specific autoantibodies; 2) association with other autoimmune diseases; 3) association with HLA haplotype; 4) lymphocytic infiltration in the site of the disease, where HLA type II antigens are expressed; 5) responsiveness to steroid therapy. In this regard, AIP is associated with the coexistence of other autoimmune diseases, hypergammaglobulinemia (IgG4), presence of several autoantibodies (Table 1), histologic evidence mainly of lymphocyte infiltration and HLA-DR expression linked with T-lymphocyte mediated apoptosis, and a favorable response to steroid therapy [3,4,6], which represent clinical evidence of autoimmunity [52]. Notably, infectious agents are considered as causative agents and contributors to lesion expression in autoimmune disease [53].

## Pathophysiology / pathogenesis of AIP (Fig. 1)

Occasional coexistence of pancreatitis with other autoimmune diseases [4-6,11] suggests that there may be common target antigens in the pancreas and other exocrine organs, such as the salivary glands, gastrointestinal or biliary tract, and renal tubules [3,4]. Several autoantibodies such as anticarbonic anhydrase II antibody (ACA-II) or antilactoferrin antibody (ALF) were frequently detected in patients with AIP, although these antibodies are not necessarily specific for AIP [3,4,6] (Table 1). Carbonic anhydrases (CA) are a family of zinc metal enzymes that catalyze the reversible hydratation of carbon dioxide to bicarbonate and hydrogen ions [49]. The enzymes are mainly distributed in the gastrointestinal tract, particularly in the salivary glands, stomach, duodenum, colon and biliary tract [4,49]. CA type II antigens are located in the pancreatic ductal epithelium. Thus, the presence of antibodies against this isoenzyme may provide evidence of an immune reaction to a pancreatic target antigen [3,4,49]. Similarly, lactoferrin (LF), a nonenzymatic protein, is also detected in various human tissues, including the lactating breast, bronchial, salivary, gastric glands or the pancreatic acinus [3,4], and the high prevalence of ALF in AIP suggests that LF may also be a candidate for the target antigen eliciting humoral and cellular-mediated immune responses in AIP [3,4].

An autoimmune reaction against CA II or LF *via* T helper (Th)-1 type CD4+ T lymphocytes might play a role in the development of AIP [6]. Experimental evidence indicates that neonatally thymectomized (NTx) BALB/c mice, subcutaneously immunized with CA II or LF, and synergetic nude mice, with splenocytes transferred from disease induced NTx-mice, developed pancreatitis early, as well as sialoadenitis and cholangitis, while the normal BALB/c mice did not [3,50]. In immunized NTx mice, the prevalence of inflammation was significantly higher in the pancreas [54]. The effector cells were found to be T lymphocytes, especially Th1 type CD4+ T lymphocytes, mostly involved in the development of pancreatitis, sialoadenitis, and cholangitis [3,50]. CA II or LF immunized mice had apoptotic duct cells or acinar cells, respectively [54]. Expression of the interferon (IFN)-γ gene was upregulated in each group [54]. Similar findings were observed in the salivary glands and liver [54]. Therefore, an immunologic mechanism against CA II or LF is involved in the pathogenesis of these pancreatitis models, in which the effector cells are mainly Th1 type CD4+ T cells [54] exhibiting apoptotic activities. These T cell-mediated responses are accompanied by mononuclear and polymorphonuclear cell infiltration in the pancreas in the first three weeks and a consequent fibrosis in the most advanced stages (6 weeks) with progressive acinar atrophy [49].

Activated CD4+ and CD8+ T cells bearing HLA-DR were increased in peripheral blood lymphocytes and the pancreas of AIP patients [3,4]. HLA-DR antigens are expressed on the pancreatic duct cells as well as on CD4+ T cells, suggesting an autoimmune mechanism involved in inflammation [3,4]. Patients with AIP have the particular HLA haplotype DRB1*0405-DQB1*0401 [50]. CD4+ T cells are subdivided into Th1 and Th2 cells based on the profiles of cytokine production. Th1 lymphocytes, which produce interleukin (IL)-2, IFN-γ and tumor necrosis factor (TNF)-α, mediate cellular immunity, macrophage activation, cytotoxicity, and stimulate B cell to produce opsonizing and complement fixing antibodies [3,4]. In contrast, Th2 lymphocytes, which produce IL-4, -5, -6 and -10, pro-

**Table 1** Characteristics of autoimmune pancreatitis [3-6,49,50]

**Demographic Indices**

- Prevalence: 4.6-6.0% of chronic pancreatitis
- Mean age: 59.1-59.4 years
- Gender: Male preponderance (male/female ratio: 2:1)

**Symptoms-Clinical Signs**

- No symptoms or only mild symptoms, frequently without acute attacks of pancreatitis
- Obstructive jaundice (70-80%)

**Radiologic Imaging**

- Diffuse enlargement of the pancreas ("sausage-like") on ultrasonography, CT, or MRI images
- Occasional tumor-like local swelling of the pancreas
- Diffuse or segmental irregular narrowing of the main pancreatic duct on endoscopic retrograde cholangiopancreatography (ERCP) images
- Rarely, pancreatic calcification or cysts

**Laboratory findings**

- Elevated levels of serum or urinary pancreatic enzymes, and CA19-9
- Abnormal pancreatic exocrine function
- Hypergammaglobulinemia or increased levels of serum IgG (37-76%)
- Presence of autoantibodies [antinuclear antibody (ANA), anti-carbonic anhydrase antibody (ACA II), antilactoferrin antibody (ALF), anti-α-fodrin antibody (AFA), rheumatoid factor (RF), antismooth muscle antibody (ASMA), antineutrophil cytoplasmic antibody (ANCA)] (10-100%)

**Histological findings**

- Fibrotic changes with infiltration of lymphocytes, plasma cells, and occasionally neutrophilic and eosinophilic granulocytes in the pancreas tissue
- Occasional associated diseases
  - Other autoimmune diseases, such as Sjögren's syndrome, primary biliary cirrhosis or sclerosing cholangitis (12-60%)
- Diabetes mellitus (type 1 and mainly type 2) (42-76%)
- Effective steroid therapy
  - Usually effective for both the pancreas and biliary tract
  - Sometimes effective for diabetes mellitus

mote humoral and allergic responses [3,4]. Th1 cytokines may be critical in the induction and/or maintenance of AIP whereas Th2 cytokines may be implicated in disease progression [4]. Some AIP patients associated with diabetes mellitus also show evidence of autoimmune diabetes (type 1) by the presence of autoantibodies against glutamic acid decarboxylase, insulin, or tyrosine phosphatase-like protein [50]. Cellular-mediated immune responses may be implicated in the pathophysiology of autoimmune diabetes induced by Th1 cell-mediated apoptosis of insulin-producing β-cells [22].

The reported clinical and animal experimental aspects might lead to the following proposed pathogenetic sequence in AIP [3]: The first step in the disease may be an antigenic alteration in pancreatic ductal or acinar cells, such as the aberrant expression of HLA-DR. In turn, CD4+ T cells may recognize the HLA class II complex and autoantigenic peptides such as CA II, and act as helper or cytotoxic cells probably by inducing apoptosis. CD8+ T cells may also act as cytotoxic cells.

This cell-mediated cytotoxic mechanism, involved in the pathogenesis of AIP mostly *via* apoptosis, appears to be reinforced by the following pathological findings observed in patients with AIP: (a) An intense inflammatory cellular infiltration mainly localizes around the medium-sized and large interlobular pancreatic ducts, but also involves the other pancreatic structures (acini, vessels, and nerves) [3,6,50]. The inflammatory infiltration consists mainly of lymphocytes and plasma cells but also contains some macrophages and occasionally neutrophilic and eosinophilic granulocytes [4-6,50]. Immunocytochemical typing of the lymphocytes reveals that the majority of them are CD4+ and CD8+ T lymphocytes, with fewer B lymphocytes [3,6,50]; (b) An increased expression of the major antigen of histocompatibility type II (HLA-DR) antigens is observed on the epithelial cells of the pancreatic ducts, which normally do not express these antigens, as well as on CD4+ T cells [3,4,6]; (c) The infiltration may be primarily subepithelial, with the epithelium only rarely being infiltrated by lymphocytes [50]. It completely encompasses the ducts and may narrow their lumen by infolding of the epithelium, often giving the lumen a starlike structure [50]. The periductal and ductal inflammation causes narrowing, obstruction and sometimes destruction of ducts. Extension of the inflammatory process to the acinar tissue leads to its replacement by fibrosis caused by such processes as necrosis/apoptosis [10], and, finally, the lobular architecture of the pancreas is almost lost [50]; (d) In a proportion of cases the chronic changes in the pancreas are overlain by "granulocytic-epithelial" lesions of the ducts [50]. This acute inflammatory component of AIP is characterized by focal detachment, disruption, and destruction of the duct epithelium due to invading neutrophilic and occasionally also eosinophilic granulocytes, which may also cluster immediately beneath the duct epithelium [50]. Occasionally, the granulocytic infiltration extends into the small intralobular ducts and acini [50]. These findings suggest that leukocyte recruitment and activation of the oxidative burst [55] may contribute to the pathophysiology of AIP; (e) T cells are involved in inducing apoptosis of acinar cells [7,56]. In particular, CD4 T cells exert direct cytotoxic effects through Fas ligand (FasL) expression [3], and FasL, TNF-α and TUNEL(+) apoptotic cells have been detected among pancreas-infiltrating cells [9]. These remarks indicate that apoptosis may be a key phenomenon in AIP.

## Apoptosis and autoimmunity

Apoptosis, often synonymously used with the term 'programmed cell death', is considered a physiological form of cell death that involves the active participation of the dying cell in its demise [24,45,57]. Although apoptosis is equally important both for the development and for the maintenance of homeostasis in some adult tissues, suppression, overexpression or mutation of a number of genes which orchestrate the apoptotic process can also be associated with disease processes [45,57,58]. Focusing on the immune system, programmed cell death is required to destroy cells that represent a threat to the integrity of the organism [57,58]. Examples include: (a) cells infected with viruses. In this situation cytotoxic T lymphocytes (CTLs) execute virus-infected cells by inducing apoptosis [45]; (b) cells of the immune system. As cell-mediated immune responses decline, the effector cells must be removed to prevent them from attacking body constituents. Therefore, CTLs induce apoptosis in each other and even in themselves, thereby main-

taining the homeostasis of the immune system [57,58]. In this respect, programmed cell death plays a key role in regulating the size of the lymphocyte pool at several stages of lymphocyte maturation and activation [57,58]. Immature lymphocytes that do not express functional antigen receptors undergo programmed death. After their maturation, if lymphocytes never encounter antigens they die by apoptosis. Even if lymphocytes are activated by antigen, fractions of the progeny that do not receive sufficient growth factors or sustained stimulation also die [57,58]. During lymphocyte maturation and activation, fluctuations in the levels of expression of Bcl-2 (acronym for the B-cell lymphoma/leukemia-2 gene) or Bcl-$x_L$ appear to correlate inversely with the susceptibility to apoptosis. Overexpression of Bcl-2 or Bcl-$x_L$ leads to enhanced survival of immature lymphocytes and prolonged antibody responses [57,58]. Conversely, knockout of Bcl-2 or BcI-$x_L$ results in reduced survival of mature or immature lymphocytes. It has also been suggested that the long life span of memory lymphocytes may be due to constitutive expression of Bcl-2 and/or Bcl-$x_L$. Unlike Bcl-2 and Bcl-$x_L$, overexpression of Bax protein induces cell death upon growth factor withdrawal [58]. These proteins have been proposed to regulate the apoptotic procedure through both homo- and heterodimerization. Notably, T lymphocytes' death involves a series of proteases (caspases), which constitute the central executioners of apoptosis [58]. Therefore, physiological regulation of cell death is essential for the removal of potentially autoreactive lymphocytes. Defects in these apoptotic mechanisms are associated with autoimmune diseases such as rheumatoid arthritis, lupus erythematosus, inflammatory bowel disease or possibly AIP [11,58]. In fact, recent work has clearly demonstrated that dysregulation of apoptosis may underlie the pathogenesis of autoimmune diseases by allowing abnormal autoreactive lymphocytes to survive, and the inappropriate accumulations of activated T cells seem to be involved in the pathogenesis and perpetuate autoimmune disorders [58] including AIP [8,11]. In addition, infectious agents (bacteria/viruses) are considered as causative agents in the induction of autoimmune diseases [53,59]. In this regard, a strong association between AIP and gastric ulcer disease has been recently documented [11]. As *H. pylori* infection is strongly associated with peptic ulceration of the stomach [23], it is reasonable to propose that *H. pylori* may act as a trigger infectious agent that contributes to the pathophysiology of AIP.

## *Helicobacter pylori*, autoimmunity and apoptosis

*Helicobacter pylori* is a gram-negative, spiral, flagellated bacterium with its large, circular chromosome comprising more than 1400 genes [23]. The bacterium is one of the most genetically diverse of all bacterial species that colonizes the gastric mucosa of most humans worldwide, mainly affecting older male adults in the developed world [60]. It has been estimated that more than one half of the world's population is infected with *H. pylori*. *Helicobacter pylori* infection is strongly associated with peptic ulceration of both the duodenum and the stomach [23]. Even though it has been early suggested that nearly all duodenal ulcers and most gastric ulcers were associated with *H. pylori* infection, more recent studies suggest that these early estimations were rather exaggerated. Nevertheless, perhaps 80% of the patients who have duodenal ulcers are infected with the organism, as are more than 60% of those with gastric ulcers [23].

In this respect, recent studies reported a high prevalence of gastric ulcer in patients with AIP [11], which also appears to affect mainly older male adults, thereby raising the possibility of an association between *H. pylori* infection and AIP [47]. This possibility is reinforced by a further association of both *H. pylori* infection and AIP with other autoimmune diseases including SjS, PBC, PSC or AIH. As in the case of AIP, these *H. pylori* related diseases are also characterized by fibrotic and/or lymphoplasmacytic inflammations [28,35-39] accompanied by aberrations of T cell apoptosis that contribute to hepatobiliary- or extrahepatic-tissue destruction [35,36,40-46]. Because *H. pylori* infection has now been implicated in the pathogenesis and pathophysiology of the above mentioned autoimmune disorders [27-31,33], this organism might also trigger AIP mainly through induction of autoimmunity and apoptosis [47].

Gastric autoimmunity is well established in patients with *H. pylori* infection associated with induction of autoantibodies that cross-react with the gastric mucosa [24,61]. The gastric $H^+/K^+$ ATPase located in canaliculi of parietal cells appears to be a target of this autoimmune response [61]. The presence of autoantibodies, in particular those directed to parietal cells, correlates with histological and clinical parameters of gastric mucosa atrophy. Therefore, *H. pylori* autoimmunity may play a critical role in the pathogenesis of chronic atrophic gastritis, a known risk factor for gastric ulceration or cancer [23,62]. It has been suggested, that molecular mimicry between *H. pylori* and the host on the level of Lewis x and y blood group antigens leads to the development of these autoantibodies [61]. *Helicobacter pylori* lipopolysacchararide (O-antigen region) expresses Lewis x and/or y blood group antigens in mimicry with human gastric epithelial cells. Mimicry may have two diverging roles in the pathogenesis of gastric mucosa injury. Infection may break tolerance and anti-Lewis antibodies may be induced to bind to gastric mucosa and cause damage probably by apoptosis. Secondly, mimicry may cause "invisibility" of the pathogen to the host, thus aiding persistence of infection [61]. It is relevant to note that *H. pylori* infection is associated with the synthesis of parietal cell autoantibodies, which, after eradication of the infection, persist and contribute to the recurrent antral chronic gastritis and intestinal metaplasia. Moreover, serum parietal cell autoantibodies are correlated with anti-*H. pylori* antibody titers [61]. Therefore, the serological titer of anti-*H. pylori* seems to reflect the autoimmunity status that correlates with gastric mucosal atrophy. This concept is further supported by the evidence indicating that the serological titer of anti-*H. pylori* IgG has been found to correlate with the histological grading of gastritis in patients with ulcer and non-ulcer dyspepsia. Thus, the titer of *H. pylori* serology may indirectly offer evidence of the severity of histological inflammatory changes [61].

Interestingly, molecular mimicry of host structures by constituents (such as the saccharide portion of lipopolysaccharides) of *H. pylori* is thought to be connected with the development of autoimmune sequelae in autoimmune neuropathies [23,61,63], PBC [28,29] or possibly AIP, that induce apoptotic damage of neurons [23,61,63], liver tissue [40,42,43], or pancreatic tissue. Support for this theory is provided by reports showing that there is a positive association between the titer of anti-*H. pylori* antibodies and the titer of anti-pyruvate dehydrogenase antibodies in patients with PBC, and *H. pylori* infection could induce autoimmune responses in the development of both PBC and atrophic corpus gastritis [29]. Of note, apoptosis is a mechanism for cell surface expression of the autoantigen pyruvate dehydrogenase complex in patients with PBC [64]. Moreover, PBC patients positive for *H. pylori* have significantly higher values of alkaline phosphatase and prothrombin complex [28], indices reflecting liver tissue destruction. The most likely mechanism for the role of this organism is via molecular mimicry autoimmune sequelae. Future studies, however, are needed to support the hypothesis that the presence of IgG antibodies to *H. pylori* may adversely influence the pathophysiology of AIP [47] and other related autoimmune diseases.

Bacterial heat shock proteins (Hsps), particularly Hsp-60 or Hsp-70 of *H. pylori*, may represent major target antigens responsible for molecular mimicry causing autoreactivity between *H. pylori* and the host's immune gastric tissue. Due to the wide homology between bacterial Hsps and the mammalian counterparts, the humoral and/or cellular (T-cell) response against these proteins has been proposed to influence the pathogenesis of autoimmune diseases [23,61]. There is evidence that the presence of increased serum autoantibodies against Hsps may have pathogenetic importance by facilitating apoptotic cell death [61]. Because Hsps are recently being discussed as promising candidates for subunit vaccines, efforts to rule out the possibility or to demonstrate that *H. pylori* Hsps can trigger autoimmune mechanisms leading to autoimmune disorders such as SjS [23,61] or possibly AIP [47], and other autoimmune and vascular disorders must be considered.

Current studies indicate that apoptosis is a mechanism of cell death in several important *H. pylori*-associated upper gastrointestinal disorders and/or extraintestinal diseases, including autoimmune entities such as SjS, PBC, PSC, AIH [40-44] and possibly AIP [47]. *Helicobacter pylori* infection is associated with significant gastric epithelial cell damage including an increased level of apoptosis [61]. It also induces apoptosis of fibroblasts and smooth muscle cells in lamina propria. These alterations may be affected by exaggerated acid secretion, decreased

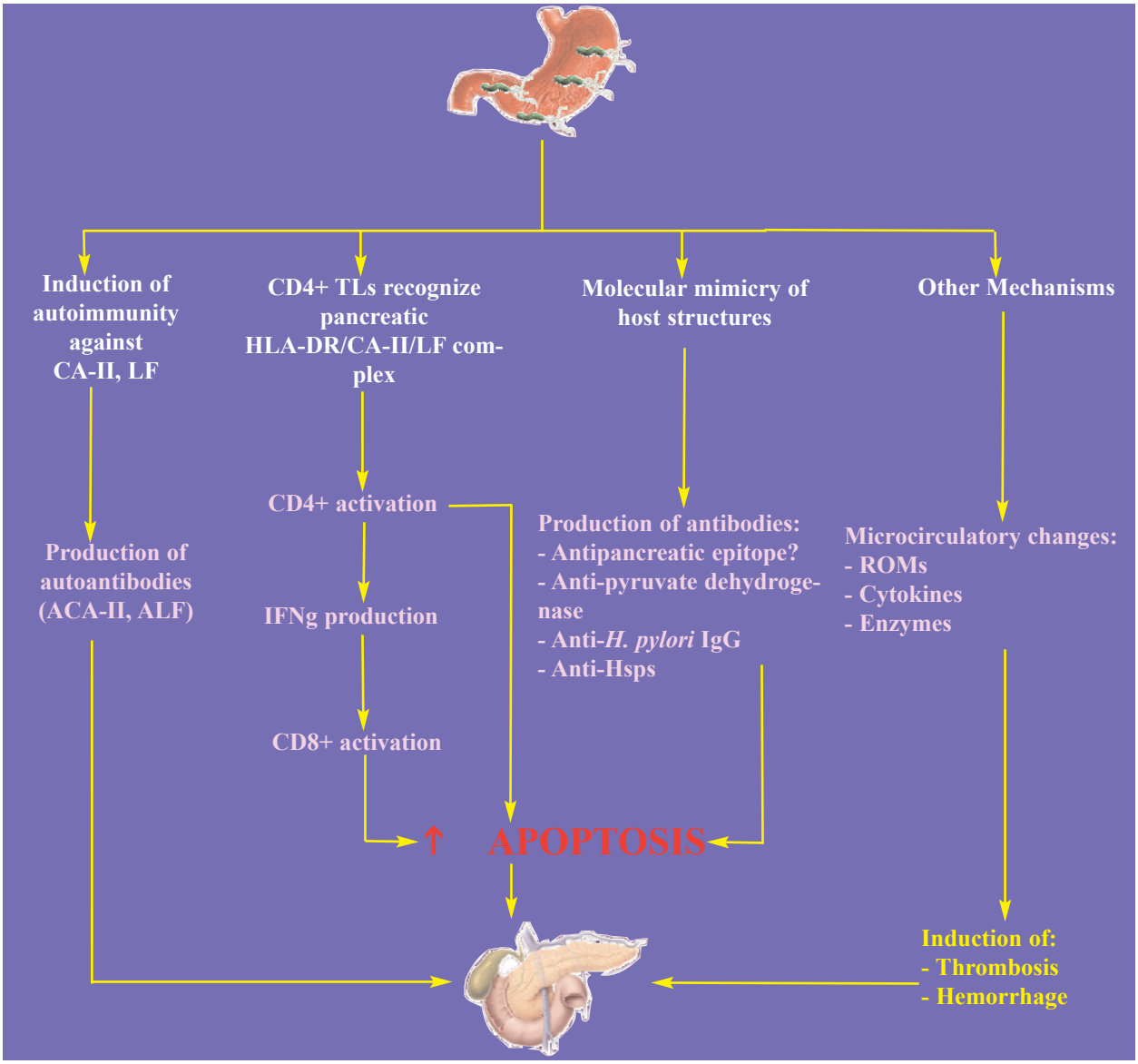


**Fig. 1** Schematic presentation of the proposed pathophysiological mechanisms by which *Helicobacter pylori* infection might contribute to autoimmune pancreatitis (CA-II, carbonic anhydrase type II antigens; ACA-II, anticarbonic anhydrase II antibody; LF, lactoferrin; ALF, antilactoferrin antibody; TLs, T lymphocytes; IFN-γ, interferon-γ; anti-Hsps, antibodies against heat shock proteins; ROMs, reactive oxygen metabolites)

mucus protecting factors, and result in ulcer formation. In particular, *H. pylori* is found to change the expression of genes encoding growth factors, cytokine/chemokines and their receptors, apoptosis proteins, transcription factors, metalloproteinase-disintegrin proteins and tissue inhibitors of metalloproteinases that contribute to the pathogen-induced gastrointestinal and extradigestive disorders [61]. *Helicobacter pylori* infection leads to injury and cellular infiltration of T lymphocytes of the gastric epithelium by inducing activation of apoptotic surface markers on human lymphocytes and gastric epithelial cells and/or gastric adenocarcinoma cell lines. Moreover, *H. pylori* appears to upregulate the expression of HLA class II (HLA-DR) molecules on gastric epithelial cells and to induce their apoptosis probably through activation of T cells [61]. Gastric epithelium, in particular, may acquire antigen-presenting cell (APC) properties in *H. pylori* infection through *de novo*

expression of HLA-DR and co-stimulatory molecules. Macrophages in the lamina propria may also act as APC in the *H. pylori*-infected gastric mucosa. *Helicobacter pylori* possesses virulent factors promoting colonization (urease) that bind to class II major histocompatibility complex on gastric epithelial cells and induce their apoptosis [61]. On the other hand, elimination of *H. pylori* is associated with attenuation of HLA-DR expression on gastric epithelial cells and remission of mucosal inflammation. Taken together, these data support the interplay between *H. pylori* and epithelial cells in the course of *H. pylori*-mediated apoptotic gastropathy [61].

There is evidence suggesting that immune-mediated gastric epithelial cell apoptosis through Fas/FasL interactions participates in *H. pylori* disease pathogenesis [61]. Fas expression is abundantly increased on fundic gland epithelium, and FasL is detected on lamina propria mononuclear cells in *H. pylori*-infected mucosa, indicating that T cell-mediated cytotoxicity *via* Fas/FasL signaling may contribute to the induction of apoptosis in gastric epithelial cells during *H. pylori* infection. In particular, wild-type *H. pylori* strains increase Fas protein expression, and *H. pylori*-induced apoptosis involves the Fas/caspase cascade. Indeed, caspases -8, -3 and -7, are activated time-dependently by *H. pylori* as well as by the agonist anti-Fas [61]. Virulence factors possessed by *H. pylori* that induce tissue damage [lipopolysaccharide (LPS)] activate Fas/FasL-mediated caspase-8 release, and moreover stimulate cytochrome c release from the mitochondria, and subsequently activate caspases -9 and -3, leading to apoptosis, thereby suggesting that caspase-8 and mitochondria may play crucial roles in *H. pylori* LPS-induced apoptosis and that this accelerated apoptosis may be involved in abnormal cell turnover of *H. pylori*-infected gastric mucosa [61]. Additional studies also suggest that *H. pylori*-induced apoptosis in gastric epithelial cells is mediated by altered expression of the products of the Bcl-2 and Bax (increased expression of proapoptotic Bax and decreased expression of antiapoptotic Bcl-2) [61]. From another point of view, recent data suggest that the water-soluble surface proteins of *H. pylori* suppress neutrophil apoptosis. This may be caused by the suppression of FasL expression in neutrophils and Fas/FasL and TNF-Receptor1 expression on the surface of neutrophils. *Helicobacter pylori* water extracts also suppress the activation of caspases -8 and -3, and upregulate the expression of antiapoptotic Bcl-xL mRNA and proteins in neutrophils. The resulted prolongation of neutrophil life span could, in turn, contribute to the pathogenesis of *H. pylori* infection [55,61]. In addition, the longer survival of polymorphonuclear leukocytes (PMNL), induced by *H. pylori* LPS that suppresses spontaneous PMNL apoptosis, may also increase gastric epithelium injury in *H. pylori*-associated diseases, since longer survival and activation of PMNL provide a major source of reactive oxygen metabolites, which can cause tissue damage mainly in the absence of antioxidants [61].

As in the case of *H. pylori* infection, comparable T cell-mediated apoptotic signals and granulocyte recruitment and activation of the oxidative burst also contribute to the pathogenesis of AIP [3,4,7,9,10,50,56]. Therefore, in the perspective of the above-mentioned data, it is reasonable to suggest that, apart from the induction of autoimmunity, *H. pylori* might trigger AIP through a variety of apoptotic signals [47].

Finally, microcirculatory changes, including vasoconstriction, capillary stasis, decreased oxygen saturation, and progressive ischemia, could lead to local microcirculatory failure, vascular permeability, edema of the gland and amplification of the pancreatic injury [65]. Apart from reactive oxygen metabolites, active granulocytes and macrophages release proinflammatory cytokines (TNF, IL -1, -6 and -8), arachidonic acid metabolites (prostaglandins, platelet-activating factor and leukotrienes), proteolytic and lipolytic enzymes; these substances also interact with the pancreatic microcirculation to augment vascular permeability, which induces thrombosis and hemorrhage and leads to pancreatic necrosis [65]. *Helicobacter pylori* infection could exacerbate these events by promoting platelet and platelet-leukocyte aggregation, releasing large amounts of proinflammatory and vasoactive substances, such endothelin-1 (a potent constrictor of arterioles and venules), cytokines (IL -1, -6, -8, TNF-$\alpha$), eicosanoids (leukotrienes, prostaglandins) or stimulating mononuclear cells to induce a tissue factor-like procoagulant activity that converts fibrinogen into fibrin [24,60,61,66].

In conclusion, we can consider that various autoimmune and apoptotic sequelae induced by *H. pylori* appear to influence the pathophysiology of

AIP, thereby suggesting an underlying link between *H. pylori* infection and AIP. If eradication of *H. pylori* infection may indirectly offer benefit to the AIP patients by ameliorating the autoimmune sequelae and the apoptotic loss of duct cells and/or acinar pancreatic cells, remains to be elucidated.

## References


1. **Sarles H., Sarles J.C., Camatte R., Muratore R., Gaini M., Guien C., Pastor J., Le Roy F.,** Observations on 205 confirmed cases of acute pancreatitis, recurring pancreatitis, and chronic pancreatitis, *Gut*, **6**: 545-559, 1965
2. **Yoshida K., Toki F., Takeuchi T., Watanabe S., Shiratori K., Hayashi N.,** Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis, *Dig. Dis. Sci.,* **40**: 1561-1568, 1995
3. **Okazaki K., Uchida K., Chiba T.,** Recent concept of autoimmune-related pancreatitis, *J. Gastroenterol.,* **36**: 293-302, 2001
4. **Okazaki K., Chiba T.,** Autoimmune related pancreatitis, *Gut,* **51**: 1-4, 2002
5. **Forsmark C.E.,** Chronic pancreatitis, In: Sleisenger & Fordtran's Gastrointestinal and Liver Disease, 7th Edn, Philadelphia, PA: Saunders, pp. 943-969, 2002
6. **Kim K., Kim M.W., Song M.H., Lee S.S., Seo D.W., Lee L.K.,** Autoimmune chronic pancreatitis, *Am. J. Gastroenterol.,* **99**: 1605-1616, 2004
7. **Hashimoto Y., Matsuoka N., Kawakami A., Tsuboi M., Nakashima T., Eguchi K., Tomioka T., Kanematsu T.,** Novel immunosuppressive effect of FK506 by augmentation of T cell apoptosis, *Clin. Exp. Immunol.,* **125**: 19-24, 2001
8. **Hill N.J., Van Gunst K., Sarvetnick N.,** Th1 and Th2 pancreatic inflammation differentially affects homing of islet-reactive CD4 cells in nonobese diabetic mice, *J. Immunol.,* **170**: 1649-1658, 2003
9. **Watanabe S., Suzuki K., Kawauchi Y., Yamagiwa S., Yoneyama H., Kawachi H., Okada Y., Shimizu F., Asakura H., Aoyagi Y.,** Kinetic analysis of the development of pancreatic lesions in mice infected with a murine retrovirus, *Clin. Immunol.,* **109**: 212-223, 2003
10. **Kloppel G., Detlefsen S., Feyerabend B.,** Fibrosis of the pancreas: the initial tissue damage and the resulting pattern, *Virchows Arch.,* **445**: 1-8, 2004
11. **Shinji A., Sano K., Hamano H., Unno H., Fukushima M., Nakamura N., Akamatsu T., Kawa S., Kiyosawa K.,** Autoimmune pancreatitis is closely associated with gastric ulcer presenting with abundant IgG4-bearing plasma cell infiltration, *Gastrointest. Endosc.,* **59**: 506-511, 2004
12. **Kawaguchi K., Koike M., Tsuruta K., Okamoto A., Tabata I., Fujita N.,** Lymphoplasmacytic sclerosing pancreatitis with cholangitis: a variant of primary sclerosing cholangitis extensively involving pancreas, *Hum. Pathol.,* **22**: 387-395, 1991
13. **Erkelens G., Vleggaar F., Lesterhuis W., Buuren H., Werf S.,** Sclerosing pancreato-cholangitis responsive to steroid therapy, *Lancet,* **354**: 43-44, 1999
14. **Kram M.T., May L.D., Cooperman A., Bernstein S., Abedeer R., Cohen P.R.,** Lymphoplasmacytic sclerosing pancreatitis and cholangitis, *Gastrointest. Endosc.,* **55**: 588-590, 2002
15. **Uchida K., Okazaki K., Asada M., Yazumi S., Ohana M., Chiba T., Inoue T.,** Case of chronic pancreatitis involving an autoimmune mechanism that extended to retroperitoneal fibrosis, *Pancreas,* **26**: 92-94, 2003
16. **Kamisawa T., Funata N., Hayashi Y., Tsuruta K., Okamoto A., Amemiya K., Egawa N., Nakajima H.,** Close relationship between autoimmune pancreatitis and multifocal fibrosclerosis, *Gut,* **52**: 683-687, 2003
17. **Horiuchi A., Kawa S., Hamano H., Ochi Y., Kiyosawa K.,** Sclerosing pancreato-cholangitis responsive to corticosteroid therapy: report of 2 case reports and review, *Gastrointest. Endosc.,* **53**: 518-522, 2001
18. **Ohana M., Okazaki K., Hajiro K., Kobashi Y.,** Multiple pancreatic masses associated with autoimmunity, *Am. J. Gastroenterol.,* **93**: 99-102, 1998
19. **Horiuchi A., Kawa S., Akamatsu T., Aoki Y., Mukawa K., Furuya N., Ochi Y., Kiyosawa K.,** Characteristic pancreatic duct appearance in autoimmune chronic pancreatitis: a case report and review of the Japanese literature, *Am. J. Gastroenterol.,* **93**: 260-263, 1998
20. **Uchida K., Okazaki K., Konishi Y., Ohana M., Takakuwa H., Hajiro K., Chiba T.,** Clinical analysis of autoimmune-related pancreatitis, *Am. J. Gastroenterol.,* **95**: 2788-2794, 2000
21. **Katamura K., Ohmori K., Chiba T.,** Autoimmune-related pancreatitis is associated with autoantibodies and a Th1/Th2-type cellular immune response, *Gastroenterology,* **118**: 573-581, 2000
22. **Pakala S.V., Kurrer M.O., Katz J.D.,** T helper 2 (Th2) T cells induce acute pancreatitis and diabetes in immune-compromised nonobese diabetic (NOD) mice, *J. Exp. Med.,* **186**: 299-306, 1997
23. **Spechler S.J.,** Peptic ulcer disease and its complications, In: Sleisenger & Fordtran's Gastrointestinal and Liver Disease, 7th Edn, Philadelphia, PA: Saunders, pp. 747-781, 2002
24. **Kountouras J., Zavos C., Chatzopoulos D.,** Induction of apoptosis as a proposed pathophysiological link between glaucoma and *Helicobacter pylori* infection, *Med. Hypotheses,* **62**: 378-381, 2004
25. **Aragona P., Magazzu G., Macchia G., Bartolone S., Di Pasquale G., Vitali C., Ferreri G.,** Presence of antibodies against *Helicobacter pylori* and its heat-shock protein 60 in the serum of patients with Sjogren's syndrome, *J. Rheumatol.,* **26**: 1306-1311, 1999
26. **Kountouras J., Deretzi G., Zavos C., Karatzoglou P., Touloumis L., Nicolaides T., Chatzopoulos D., Venizelos I.,** Association between *Helicobacter pylori* infection and acute inflammatory demyelinating polyradiculoneuropathy, *Eur. J. Neurol.,* **12**: 139-143, 2005

27. **Nilsson H.O., Castedal M., Olsson R., Wadstrom T.,** Detection of *Helicobacter* in the liver of patients with chronic cholestatic liver diseases, *J. Physiol. Pharmacol.,* **50**: 875-882, 1999
28. **Nilsson H.O., Taneera J., Castedal M., Glatz E., Olsson R., Wadstrom T.,** Identification of *Helicobacter pylor*i and other *Helicobacter* species by PCR, hybridization, and partial DNA sequencing in human liver samples from patients with primary sclerosing cholangitis or primary biliary cirrhosis, *J. Clin. Microbiol.,* **38**: 1072-1076, 2000
29. **Dohmen K., Shigematsu H., Miyamoto Y., Yamasaki F., Irie K., Ishibashi H.,** Atrophic corpus gastritis and *Helicobacter pylori* infection in primary biliary cirrhosis, *Dig. Dis. Sci.,* **47**: 162-169, 2002
30. **Leong R.W., Sung J.J.**, Review article: *Helicobacter* species and hepatobiliary diseases, *Aliment. Pharmacol. Ther.,* **16**: 1037-1045, 2002
31. **Nilsson I., Kornilovs'ka I., Lindgren S., Ljungh A., Wadstrom T.,** Increased prevalence of seropositivity for non-gastric *Helicobacter* species in patients with autoimmune liver disease, *J. Med. Microbiol.,* **52(Pt 11)**: 949-953, 2003
32. **Masaki N., Hayashi S.,** Autoimmune liver disease complicating Sjogren's syndrome [Article in Japanese], *Nippon Rinsho,* **53**: 2530-2535, 1995
33. **Dore M.P., Realdi G., Mura D., Graham D.Y., Sepulveda A.R.,** *Helicobacter* infection in patients with HCV-related chronic hepatitis, cirrhosis, and hepatocellular carcinoma, *Dig. Dis. Sci.,* **47**: 1638-1643, 2002
34. **Cammarota G., Cianci R., Grillo R.L., Martini M., Di Campli C., Pompili M., Pignataro G., Cuoco L., De Lorenzo A., Gasbarrini G., Pandolfi F., Larocca L.M.,** Relationship between gastric localization of hepatitis C virus and mucosa-associated lymphoid tissue in *Helicobacter pylor*i infection, *Scand. J. Gastroenterol.,* **37**: 1126-1132, 2002
35. **Czaja A.J.,** Autoimmune liver disease, In: Zakim D., Boyer T.D., (eds), Hepatology: A Textbook of Liver Disease, 4th Edn, Philadelphia, PA: Saunders, pp. 1163-1202, 2002
36. **Fox C.K., Furtwaengler A., Nepomuceno R.R., Martinez O.M., Krams S.M.,** Apoptotic pathways in primary biliary cirrhosis and autoimmune hepatitis, *Liver,* **21**: 272-279, 2001
37. **Talwalkar J.A., Lindor K.D.,** Primary biliary cirrhosis, In: Zakim D., Boyer T.D., (eds), Hepatology: A Textbook of Liver Disease, 4th Edn, Philadelphia, PA: Saunders, pp. 1203-1220, 2002
38. **Vierling J.M.,** Hepatobiliary complications of ulcerative colitis and Crohn's disease, In: Zakim D., Boyer T.D., (eds), Hepatology: A Textbook of Liver Disease, 4th Edn, Philadelphia, PA: Saunders, pp. 1221-1272, 2002
39. **Hoofnagle J.H., Heller T.,** Hepatitis C, In: Zakim D., Boyer T.D., (eds), Hepatology: A Textbook of Liver Disease, 4th Edn, Philadelphia, PA: Saunders, pp. 1017-1062, 2002
40. **Yeaman S.J., Kirby J.A., Jones D.E.,** Autoreactive responses to pyruvate dehydrogenase complex in the pathogenesis of primary biliary cirrhosis, *Immunol. Rev.*, **174**: 238-249, 2000
41. **Kita H., Mackay I.R., Van De Water J., Gershwin M.E.,** The lymphoid liver: considerations on pathways to autoimmune injury, *Gastroenterology*, **120**: 1485-1501, 2001
42. **Deguchi A., Arima K., Masaki T., Yachida M., Nakai S., Ito T., Kita Y., Kurokohchi K., Watanabe S., Kuriyama S.,** Enhanced expression of Bcl-2 in lymphocytes infiltrating into the liver of patients with primary biliary cirrhosis, *Int. J. Mol. Med.*, **9**: 571-577, 2002
43. **Tinmouth J., Lee M., Wanless I.R., Tsui F.W., Inman R., Heathcote E.J.,** Apoptosis of biliary epithelial cells in primary biliary cirrhosis and primary sclerosing cholangitis, *Liver*, **22**: 228-234, 2002
44. **Ishibashi H., Nakamura M., Shimoda S., Gershwin M.E.,** T cell immunity and primary biliary cirrhosis, *Autoimmun. Rev*., **2**: 19-24, 2003
45. **Kountouras J., Zavos C., Chatzopoulos D.,** Apoptosis in hepatitis C, *J. Viral Hepat.,* **10**: 335-342, 2003
46. **Kita H., Imawari M., Gershwin M.E.,** Cellular immune response in primary biliary cirrhosis, *Hepatol. Res.,* **28**: 12-17, 2004
47. **Kountouras J., Zavos C., Chatzopoulos D.,** Autoimmune pancreatitis, *Helicobacter pylori* infection and apoptosis: a proposed relationship, *Pancreas*, **30**: 192-193, 2005
48. **Etemad B., Whitcomb D.C.,** Chronic pancreatitis: diagnosis, classification, and new genetic developments, *Gastroenterology*, **120**: 682-707, 2001
49. **Cavallini G., Frulloni L.,** Autoimmunity and chronic pancreatitis: a concealed relationship, *J.O.P.*, **2**: 61-68, 2001
50. **Kloppel G., Luttges J., Lohr M., Zamboni G., Longnecker D.,** Autoimmune pancreatitis: pathological, clinical, and immunological features, *Pancreas*, **27**: 14-19, 2003
51. **Snook J.,** Are the inflammatory bowel diseases autoimmune disorders?, *Gut*, **31**: 961-963, 1990
52. **Mackay I.R., Gershwin M.E.,** The nature of autoimmune disease, *Semin. Liver Dis.,* **17**: 3-11, 1997
53. **Rouse B.T., Deshpande S.,** Viruses and autoimmunity: an affair but not a marriage contract, *Rev. Med. Virol.,* **12**: 107-113, 2002
54. **Uchida K., Okazaki K., Nishi T., Uose S., Nakase H., Ohana M., Matsushima Y., Omori K., Chiba T.,** Experimental immune-mediated pancreatitis in neonatally thymectomized mice immunized with carbonic anhydrase II and lactoferrin, *Lab. Invest.,* **82**: 411-424, 2002
55. **Kountouras J., Zavos C., Chatzopoulos D.,** Reactive oxygen metabolites and upper gastrointestinal diseases, *Hepatogastroenterology*, **48**: 743-751, 2001
56. **Yamada T., Hashimoto T., Sogawa M., Kobayashi S., Kaneda K., Nakamura S., Kuno A., Sano H., Ando T., Kobayashi S., Aoki S., Nakazawa T., Ohara H., Nomura T., Joh T., Itoh M.,** Role of T cells in development of chronic pancreatitis in male Wistar Bonn/Kobori rats: effects of tacrolimus, *Am. J. Physiol. Gastrointest. Liver Physiol.,* **281**: G1397-G1404, 2001
57. **Kountouras J., Zavos C., Chatzopoulos D.,** Apoptosis

in hepatocellular carcinoma, *Hepatogastroenterology,* **50**: 242-249, 2003

58. **Kountouras J., Kouklakis G., Zavos C., Chatzopoulos D., Moschos J., Molyvas E., Zavos N.,** Apoptosis, inflammatory bowel disease and carcinogenesis: overview of international and Greek experiences, *Can. J. Gastroenterol.,* **17**: 249-258, 2003
59. **Blank M., Shoenfeld Y.,** Beta-2-glycoprotein-I, infections, antiphospholipid syndrome and therapeutic considerations, *Clin. Immunol.,* **112**: 190-199, 2004
60. **Kountouras J., Mylopoulos N., Chatzopoulos D., Zavos C., Boura P., Konstas A.G.P., Venizelos J.,** Eradication of *Helicobacter pylori* may be beneficial in the management of chronic open-angle glaucoma, *Arch. Intern. Med.,* **162**: 1237-1244, 2002
61. **Kountouras J., Zavos C., Chatzopoulos D.,** Apoptosis: an overview and a proposed link between glaucoma and *Helicobacter pylori* infection, In: Reece S.M. (ed), Trends in Glaucoma Research, New York: Nova Science Publishers, 2005 (in press)
62. **Koh T.J., Wang T.C.,** Tumors of the stomach, In: Sleisenger & Fordtran's Gastrointestinal and Liver Disease, 7th Edn, Philadelphia, PA: Saunders, pp. 829-855, 2002
63. **Kountouras J., Mylopoulos N., Konstas A.G., Zavos C., Chatzopoulos D., Boukla A.,** Increased levels of *Helicobacter pylori* IgG antibodies in aqueous humor of patients with primary open-angle and exfoliation glaucoma, *Graefes Arch. Clin. Exp. Ophthalmol.,* **241**: 884-890, 2003
64. **Macdonald P., Palmer J., Kirby J.A., Jones D.E.,** Apoptosis as a mechanism for cell surface expression of the autoantigen pyruvate dehydrogenase complex, *Clin. Exp. Immunol.,* **136**: 559-567, 2004
65. **DiMagno E.P., Chari S.,** Acute pancreatitis, In: Sleisenger & Fordtran's Gastrointestinal and Liver Disease, 7th Edn, Philadelphia, PA: Saunders, pp. 913-941, 2002
66. **Kountouras J., Mylopoulos N., Boura P., Bessas C., Chatzopoulos D., Venizelos J., Zavos C.,** Relationship between *Helicobacter pylori* infection and glaucoma, *Ophthalmology*, **108**: 599-604, 2001