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# Autoimmune Gastric Disease Chemical and Clinical Studies with Special Reference to $H^+$, $K^+$-ATPase

*Minireview based on a doctoral thesis*

Pia Burman

*Department of Internal Medicine, University Hospital, Uppsala, Sweden*

## INTRODUCTION

### Pernicious anemia; a historical review

In the London Medical Gazette, Dr Thomas Addison in 1849 pointed out an unusual form of anemia of idiopathic origin which in his view had not attracted the attention it deserved, although occasionally noticed by others. He described three men with insidious anemia who, upon postmortem examination, all were found to have 'a diseased condition of the suprarenal capsules' (2). He considered this observation to be of relevance to the anemia. This was questioned by Dr Austin Flint, who himself had experience of such patients and who suspected the existence of a degenerative disease of the gastric tubular glands. At a clinical lecture at the Long Island College Hospital in 1860, he referred to an article by Dr Handfield Jones, who some years earlier had published his findings on microscopic examinations of a hundred stomachs. In 14 cases considerable degeneration of the gastric tubuli was noted. Dr Flint stated: 'It is not difficult to see how fatal anemia must follow an amount of degenerative disease reducing the amount of gastric juice so far that the assimilation of food is rendered wholly inadequate to the wants of the body. I shall be ready to claim the merit of this idea when the difficult and laborious researches of some one have shown it to be correct' (39). Seventeen years later, in certain patients with anemia, Fenwick noted atrophic glands and failure of the scrapings of the gastric mucosa to digest egg white in autopsy studies (36), and during the following decades, by analysis of the amount of 'free acid' in the gastric juice of patients with pernicious anemia, several investigators observed achylia to be a constant finding (24).

The name pernicious anemia was adopted in England in 1874 from a description by Biermer in 1872 of patients with severe forms of anemia (9). Pernicious means fatal and refers to the pessimistic prognosis of the disease at that time. Before the introduction of specific therapy, the disease was characterized by a slow downhill course with remissions and relapses. The deterioration was often precipitated by a change in diet, with an increased consumption of dairy products such as butter and cream and a distaste for meat (24). Three organ systems were affected to a varying extent - the blood, the digestive tract, and the nervous system. On presentation the patients generally complained of a sore tongue, weakness and symmetrical paresthesias of the hands and feet. In about 30 %, more severe degenerative changes of the lateral and dorsal columns of the spinal cord and/or the cerebral cortex had developed and led to ataxia, a spastic gait, atony of the bladder and incontinence and even mental confusion (153). In fact Hurst stated that if carefully looked for about 80 % of all patients with pernicious anemia would be found to have minor signs of spinal cord disease (53). Sturgis described the episodic painful glossitis that was present in about two thirds of the patients (142). The entire dorsum of the tongue was usually affected, leading to difficulties in swallowing and subsequently to atrophy of the papillae, giving the tongue a smooth appearance. As for the blood, the anemia was long considered to be of hemolytic origin. Hurst hypothesized that an abnormal gut flora, favored by the anacidic environment, had developed with the production of hemolytic (and neurotoxic) toxins, and advocated administration of diluted hydrochloric acid to the patients (53).

During the early part of the 20th century many different diets were recommended to anemic patients, including fresh vegetables, large amounts of proteins and, especially, blood products and even fresh bone marrow. Whipple and associates (151) systematically investigated the effects of various food contents on the blood formation after acute hemorrhage in dogs, and found that liver was particularly effective. This inspired Minot and Murphy to treat 45 patients with pernicious anemia with a special diet consisting of about 200 grams of cooked calf's or beef liver per day. A rapid and distinct response on the red blood cell count and clinical condition was observed (97). An even better response was noted when extracts of liver, diluted in water, were given orally and parenterally (96, 98). Minot, Murphy and Whipple were awarded with the Nobel Prize for this discovery in 1934. William Castle, a student of Minot's, concentrated on the loss of an 'intrinsic factor' of the gastric mucosa in patients with gastric atrophy and pernicious anemia. He found that patients benefited from administration of normal gastric juice in combination with beef muscle (referred to as extrinsic factor) and that the anemia could be reversed in

the same way as if liver had been administered. The intrinsic factor of gastric juice was separate from hydrochloric acid and pepsin and was heat labile (15, 16).

Vitamin $B_{12}$ was isolated from liver in 1948 (108, 126) and was found to be identical to the hematopoietic factor of liver extracts. The molecule was characterized by x-ray diffraction studies in 1956 by Dorothy Hodgkin (51), an achievement for which she received a Nobel Prize eight years later. Low levels of vitamin $B_{12}$ were subsequently observed in sera of patients with pernicious anemia (111). Castle's intrinsic factor was later found to be a glycoprotein with a molecular weight of around 45,000 (3). In the early 1950s there were several reports on decreased absorption of radiolabelled vitamin $B_{12}$ ($B_{12}Co^{60}$) in patients with pernicious anemia. Robert F Schilling found that normal gastric juice given simultaneously with oral vitamin $B_{12}Co^{60}$ enhanced the urinary recovery of vitamin $B_{12}Co^{60}$ and designed the test which has been given his name (120).

The first evidence that pernicious anemia is an autoimmune disease was provided by Schwartz (121) and by Taylor (142), who found inhibitors of intrinsic factor in the sera of patients with this form of anemia. The studies were prompted by the problem of acquired resistance to oral treatment with intrinsic factor (74, 121). In 1960, the inhibiting factor was found to be an immunoglobulin and to be present in the sera of 36 out of 91 patients (122). In vitro methods for detection of the antibody soon followed (6, 67). Two types of intrinsic factor antibodies, reactive to the vitamin $B_{12}$ binding site of intrinsic factor (type I antibody or blocking antibody) and to the intrinsic factor-$B_{12}$ complex (type II antibody or binding antibody), were identified (1, 6, 67, 109). The frequencies of the blocking and binding antibody have been reported to be up to 70 % and 34 %, respectively, (119), although others have found intrinsic factor antibodies in a lower frequency (60, 143, 147). Type II antibody was never observed in the absence of type I antibody (137). A second type of autoantibody reactive to parietal cells was discovered shortly afterwards (62, 91) and an immunofluorescence method, staining the cytoplasm of parietal cells, was developed (59, 143). Parietal cell antibodies were demonstrated in 75-86 % of sera (34, 60, 119). Gastric autoantibodies of both IgG and IgA subclasses have also been found in the gastric juice (38, 66, 110, 137). Strickland reported that 15 out of 20 patients with serum parietal cell antibodies also had the antibody in the gastric juice. While the IgG subclass dominated in the serum (5/20 also had IgA and 1 also IgM), the IgA subclass was more common in the gastric juice (3 had IgG only, 6 had both IgG and IgA and 6 had IgA only).

## The parietal cell

The gastric parietal cell has a unique ability to secrete $H^+$ against a two million-fold concentration gradient. As this is an energy-consuming process, more than a third of the cell volume is occupied with mitochondria, a higher proportion than in any other epithelial cell (49). In the resting state the cytoplasm is filled with numerous smooth tubulovesicular structures, as well as narrow canals with microvilli that are referred to as secretory canaliculi. Upon stimulation of the parietal cell, the secretory canaliculi expand to an extensive network and the microvilli become elongated and numerous. There is concomitant loss of tubulovesicular structures (Fig 1). The secretory surface, i.e. the total membrane area facing the luminal side, is thus expanded six- to ten-fold compared with the resting condition. This change is reversed when the stimulus is withdrawn (50, 58). It is well established that $H^+,K^+$-ATPase is the primary enzyme responsible for proton secretion in the stomach. With the use of a monoclonal antibody to $H^+,K^+$-ATPase, more intense staining of the tubulovesicles was observed during resting states, while the apical cell surface stained heavily after stimulation (128). Similarly, in membrane preparations from non-secreting stomachs the $H^+,K^+$-ATPase activity was mainly found in the tubulovesicles, whereas in the stimulated stomach the $H^+,K^+$-ATPase was redistributed to heavier membrane fractions, thought to be derived from the apical cell membrane (155). Compared with resting glands, vesicles isolated from homogenates of stimulated glands have an increased permeability to potassium ions (149). According to a membrane-recycling hypothesis, during stimulation tubulovesicles fuse with the apical plasma membrane, which thus recruits all the necessary structures for HCl secretion (40). Whether the translocation of the $H^+,K^+$-ATPase is accompanied by changes in the conformation of the protein structure, or whether fusion adds other proteins to the secretory canaliculi, necessary for transport of $K^+$ and $Cl^-$ ions, remains to be clarified.

## Parietal cell receptors and second messengers

Stimulation of gastric acid secretion is mediated by three compounds, a) histamine, which is released from endocrine cells (ECL cells) and/or mast cells in the oxyntic mucosa and thus acts through a paracrine route, b) acetylcholine which is released from postganglionic vagal nerve fibers in the stomach wall, and c) gastrin, which is secreted into the blood by the antral G cells (115, 154). After a meal all these pathways are in operation and potentiate each other. For instance vagal stimulation enhances gastrin release, partly by inhibition of somatostatin (157), a peptide that blocks gastrin release.

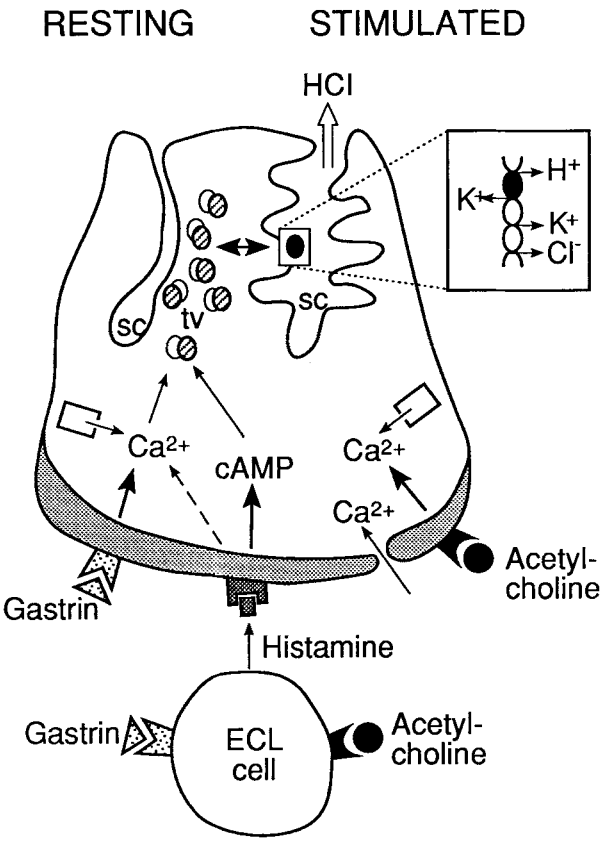


**Fig 1.** Parietal cell receptors and second messengers [modified from Sensaluu, with the kind permission of the author (123)]. In the resting state the $H^+,K^+$-ATPase (shaded circles), is located in the tubulovesicles (tv), and following stimulation the enzyme is incorporated into the secretory canaliculi (sc). This translocation allows the hydrogen ions to be pumped from the cytosol into the lumen in exchange for potassium ions. Black circles = activated $H^+,K^+$-ATPase.

Histamine plays an essential role in the stimulation of the parietal cell (130) and a receptor, blocked by $H_2$-receptor antagonists (132), has been identified on the parietal cell in all mammals investigated, including man (80, 102). Histamine stimulation activates a membrane-associated enzyme, adenylate cyclase, which catalyzes the formation of cyclic adenosine monophosphate (cAMP) from ATP (Fig 1). cAMP serves as a second messenger which modulates the function of protein kinases, which in turn phosphorylate target proteins (20, 90). Histamine can also increase the level of $Ca^{2+}$ in the cytosol and thereby modulate the secretory response (84). Prostaglandin analogues, used in the treatment of peptic ulcer disease, inhibit the histamine-stimulated activation of adenylate cyclase by binding to an inhibitory G protein associated with a subunit of adenylate cyclase (19). Besides binding to their respective receptors on the parietal cell, both acetylcholine and gastrin stimulate gastric acid secretion by release of histamine from ECL cells (154). On incubation of isolated and purified parietal cells with gastrin alone, weak responses have been obtained in the pig (104) and rabbit (21) and in man (102). It has therefore been suggested that gastrin stimulates HCl secretion mainly via histamine release. Direct binding of gastrin to an enriched parietal cell fraction was first shown in dogs (133) and has subsequently also been demonstrated in other species. Further, the presence of gastrin in the incubation medium augments the response of isolated rat parietal cells to histamine stimulation (12). Other peptides such as somatostatin and epidermal growth factor (EGF) also influence the parietal cell response. Recent data indicate that EGF inhibits histamine-stimulated secretion through activation of the inhibitory G protein associated with adenylate cyclase (81). Somatostatin directly inhibits both the parietal cell function and the release of histamine from histamine-storing cells (105).

## $H^+,K^+$-ATPase

The $H^+,K^+$-ATPase is a membrane-spanning enzyme that exchanges luminal $K^+$ for cytoplasmic $H^+$ in the presence of ATP (44, 79, 116) (Fig 2). When the parietal cells are stimulated to secrete acid, the $H^+,K^+$-ATPase is translocated to the secretory canaliculi, and the membrane becomes more permeable to potassium and chloride. This allows these ions to diffuse passively into the canalicular lumen through specific channels (31, 156). Once in the lumen the potassium ions can be pumped back in exchange for hydrogen ions. During the catalytic cycle the $H^+,K^+$-ATPase is converted to an intermediary phosphoenzyme (113), and

the intracellular regions to which the hydrogen ion is bound changes shape and is exposed to the extracellular side where the hydrogen ion is released and the potassium ion is bound (115).

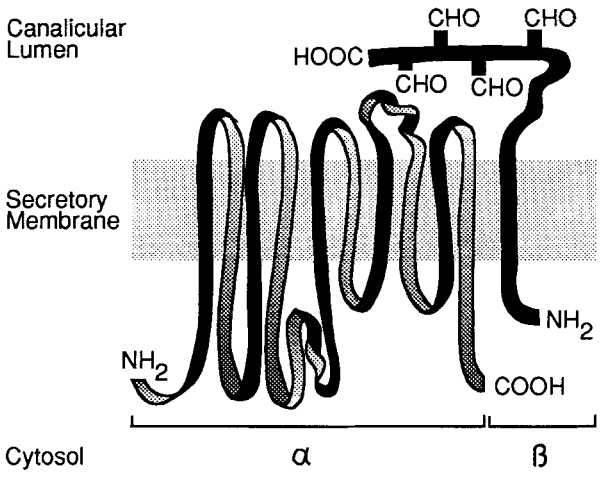


**Fig 2.** Diagram of the two protein subunits of the $H^+,K^+$-ATPase. [From Sachs and Hersey (115), with the kind permission of Börje Wernersson, Astra Hässle AB, Sweden].

It has recently been found that the enzyme consists of a protein complex that in addition to the catalytic polypeptide (now termed α subunit) contains a second peptide, β subunit (see below). The catalytic α subunit is a single peptide which migrates as a 92-95 kDa protein on SDS-PAGE (41, 85, 134). Omeprazole, which specifically binds to $H^+,K^+$-ATPase (41, 86), is a lipophilic weak base with a pKa of 4.0. In compartments with a pH less than 4.0, omeprazole is protonated and hence trapped. Under acidic conditions the compound is labile and transformed to a sulfenamide, which covalently binds to the SH groups of the cysteine residues on the luminal side of the catalytic subunit of $H^+,K^+$-ATPase, and thereby inhibits the enzyme. The resumption of acid secretion is dependent on synthesis of new protein (55, 82).

The amino acid sequence of the catalytic unit of $H^+,K^+$-ATPase was first determined in rats in 1986. The protein was found to consist of 1033 amino acid residues, Mr 114,012, and had an overall sequence homology of 62 % with the catalytic subunit of the related $Na^+,K^+$-ATPase (125). The transmembrane and the aminoterminal regions, which are possibly involved in cation discrimination, differed most extensively. The pig gastric $H^+,K^+$-ATPase contains 1034 amino

acids and has a 63 % sequence homology with pig $Na^+,K^+$-ATPase. In the amino terminal part, rich in lysine for both proteins, clusters of glycine are present in $H^+,K^+$-ATPase (88). The human gene, cloned 1990, has 22 exons and codes for a protein of 1035 residues. Of the residues, 1015 and 1013, respectively, are identical to the pig and rat enzymes (89, 103).

The $Na^+,K^+$-ATPase, which belongs to the same family of ATPases as $H^+,K^+$-ATPase, consists of two subunits, the catalytic $\alpha$ subunit and the $\beta$ subunit. The $\beta$ subunit was previously thought to be unique for the $Na^+,K^+$-ATPase. In 1990, however, several groups proposed that the proton pump also possessed a $\beta$ subunit. This subunit stained only faintly with Coomassie blue on SDS-polyacrylamide gels and was found to be rich in glycoproteins. The work of the different groups is summarized briefly in the following. Callaghan et al (13) showed binding to parietal cells of a poly-N-acetyl-lactosamine-specific tomatolectin with a pattern indicative of an intracellular membrane network. By lectin blotting, a protein of 60-90 kDa was identified in a gastric membrane preparation, which after treatment with N-glycanase migrated as a sharp 35 kDa protein, i.e. of a size similar to that of the $\beta$ subunit of $Na^+,K^+$-ATPase. Okamoto et al (106) isolated glycoproteins of 60-80 kDa from solubilized pig gastric membranes by wheat germ agglutinin affinity chromatography. The proteins were copurified with and immunoprecipitated with the 94 kDa protein. Characterization of the 32 kDa core protein showed 5 asparagine-linked oligosaccharide chains. The 35 kDa core protein of pig gastric mucosa was purified and the amino acid sequence partly determined (48, 145). At least 30 % homology with the $Na^+,K^+$-ATPase $\beta$ subunit was found. Toh et al also demonstrated colocalizatition of the two subunits to the membranes of the parietal cells by the use of monoclonal antibodies (145). Reuben et al (107) and Shull (124) determined the sequence of the $\beta$ subunit from isolated rabbit and rat cDNA clones. A single membrane-spanning portion was predicted. In the rat the protein has 294 amino acids. The cystein residues located on the luminal side do not bind omeprazole (107). The function of the $\beta$ subunit remains unknown. A $H^+,K^+$-ATPase similar but not identical to the gastric $H^+,K^+$-ATPase, has recently been identified in the rectum, and in the crypts of the transverse and descending colon in the rabbit (140).

## Autoimmune gastritis

Autoimmune gastritis is characterized by the presence of serum parietal cell autoantibodies, infiltration of mononuclear cells and loss of specialized cells of the oxyntic glands. The antrum is usually spared or only mildly affected (136).

Hypergastrinemia is present in most cases (43, 94), conceivably because of the integrity of the antral mucosa (135, 138). The loss of antral acidification with consequent loss of inhibition of gastrin release has been implicated as the main mechanism of hypergastrinemia. The relation may be indirect, however, since in a recent publication an undefined factor present in the gastric juice of patients with pernicious anemia, was shown to cause gastrin release when instilled into the stomachs of rats (32). Parietal cell antibodies are found in the sera of 70-95 % of patients with pernicious anemia, but less frequently, 30-60 %, in chronic atrophic gastritis without pernicious anemia. Antibodies to intrinsic factor, which are reported to be present in 40-75 % of patients with pernicious anemia, are rare in simple atrophic gastritis (18, 72). Some of the discrepancies in the results might be explained by patient selection, since the integrity of the gastric mucosa as well as the extension of the inflammatory process seem to influence the outcome. Thus, Korman et al (76) found parietal cell antibodies (PCA) in all 17 patients who had hypergastrinemia and concomitant endocrine autoimmune diseases and, in addition, intrinsic factor antibodies (IFA) in seven of the 17 patients. No gastric autoantibodies were found in 11 elderly patients with gastric atrophy and less elevated serum gastrin, nor in six patients with normal serum gastrin and multifocal gastritis with achlorhydria. Te Velde et al (144) compared 69 subjects with PCA, of whom the majority had achlorhydria, with 33 PCA-negative achlorhydric subjects. In the PCA-positive group the inflammatory process had a diffuse localization, in contrast to the multifocal gastritis typical of the PCA-negative group. Coghill et al (22) screened for gastric autoantibodies among 38 patients who presented with hypochromic anemia and who had atrophic body gastritis of varying degrees of severity. Twelve of the subjects had vitamin $B_{12}$ malabsorption. IFA was absent in the sera of the 38 patients, the prevalence of PCA correlated to the severity of mucosal damage, and PCA was more common in achlorhydric subjects.

## Experimental models

Circulating parietal cell antibodies, achlorhydria and degeneration of oxyntic glands have been induced in various species after immunization with homologous and autologous gastric juice or mucosal extracts (5, 78). Achlorhydria and glandular atrophy developed in dogs after repeated month-long injections of serum from rabbits which had been immunized with a microsomal canine parietal cell fraction. The rabbit anti-canine serum specifically stained the cytoplasm of the parietal cells (150). Similarly, passive transfer of immunoglobulins containing parietal cell antibodies from patients with pernicious anemia to rodents resulted in hypochlorhydria and a significant

reduction of the parietal cell mass and mucosal thickness (141). These data suggested that parietal cell antibodies might be implicated in the pathogenesis of atrophic gastritis. An exaggerated T cell response, assessed as lymphokine production or lymphocyte transformation of peripheral blood lymphocytes in the presence of gastric mucosal components, has been found in 20-40 % of patients with pernicious anemia (17, 146), although occasionally higher figures have been reported when intrinsic factor was used as the antigen (37). Ito et al (56) studied the in vivo aggregation of lymphoid cells in the skin in response to autologous gastric mucosa. A coverslip carrying cryostat sections of fundic or antral biopsies was mounted on a small abraded area of the forearm, and the cell response was studied. About 33 % of patients with moderate or severe gastritis of the body mucosa were considered positive. The figure was less striking in patients with antral gastritis.

Autoimmune gastritis accompanied by mild macrocytic anemia has been induced in BALB/c nu/+ mice by neonatal thymectomy three days after birth (75). Spleen cells, but not serum, from the thymectomized animals caused autoimmune gastritis when transferred to nude BALB/c mice. A delayed-type hypersensitivity reaction to parietal cell-enriched, but not chief cell-enriched fractions of gastric cells, was observed in the affected animals (42). Neonatal thymectomy was found to result in the disappearance of a T cell subset (Lyt-$1^+$) from peripheral lymphoid organs (117). Similarly, spleen cells from normal adult mice freed of such T cells induced endocrine organ-specific autoimmune diseases, including autoimmune gastritis, when transferred to BALB/c nude mice (118). Interestingly, parietal cell antibodies from BALB/c mice with induced autoimmune gastritis seem to recognize antigenic epitopes similar to parietal cell autoantibodies from patients with autoimmune gastritis and pernicious anemia (68, 99).

## The parietal cell antigen

Bauer et al (7) localized the parietal cell antigen to the microsomes of homogenized gastric mucosa, and in 1970 Hoedemaeker and Ito demonstrated specific binding of parietal cell antibodies to the secretory canaliculi of the parietal cells (52). Immunoglobulins from patients with pernicious anemia have been shown to inhibit spontaneous and gastrin-stimulated gastric acid secretion from the dissected stomach of a bullfrog (87). Also, passive transfer of patient immunoglobulins to rats has been found to decrease gastric acid secretion and subsequently to reduce the parietal cell mass, with thinning of the gastric mucosa (141). Parietal cell antibodies reactive to the cytoplasm of the parietal cells have

also been found to react with the cell surface of such living cells (93). Data favoring the idea that cytoplasmic and cell-surface antigens are two separate antigens came from an Australian group (26), who also provided evidence for a complement-dependent cytotoxic effect of the autoantibodies (27). The same group also reported the finding of a blocking immunoglobulin, present in about 50 % of sera from patients with pernicious anemia, that specifically inhibited the gastrin-stimulated gastric acid formation in isolated gastric mucosal cells (28). HCl production was assessed as the uptake of a weak base, $^{14}$C-aminopyrine, into acid cell compartments, a technique first described by Berglindh et al (8). By the use of a radioreceptor assay de Aizpurua et al (29) found that six out of 20 sera from patients with pernicious anemia blocked the binding of gastrin to rat gastric mucosal cells and proposed that the gastrin receptor is an autoantigen. However, they, like others, later found the gastrin receptor to be a 78 kDa protein (100), opposing their previous hypothesis. Thus, the nature of the parietal cell antigen(s) still remained elusive. Its characterization was the aim of the present work.

## RESULTS AND COMMENTS

### *The discovery of H,$^+$K$^+$-ATPase as the major autoantigen in autoimmune gastritis*

In the first study it was found that a membrane fraction of porcine gastric mucosa was 100 times more potent than a lysate of gastric mucosa in binding immunoglobulins of patients with pernicious anemia. Further, preincubation of the antibodies with the membrane fraction blocked more than 90 % of the antibody binding to the lysate. When the proteins of the two sources were separated by electrophoresis in a sodium dodecyl sulfate (SDS)-polyacrylamide gel, the major component of the gastric membrane fraction was found to be a 92 kDa protein. Upon reduction and alkylation this component was resolved into two sharp bands of similar staining intensity, with molecular weights of 88 K and 92 K. After transferring the proteins to nitrocellulose membranes, antigenic bands were identified by immunoblotting. The 88 kDa and 92 kDa proteins were equally recognized by patient sera. A broad, blurred band of 65-75 kDa was also seen in some immunoblotting experiments with non-reduced material (Fig 3). This protein was not visible on staining with Coomassie blue. In studies of our own and by others it had previously been demonstrated that the 92 kDa protein dominating the unreduced membrane fraction corresponded to $H^+$,$K^+$-ATPase, the gastric proton pump (85, 114). To further prove identity between the parietal cell antigen and the $H^+$,$K^+$-ATPase, the membrane fraction was solubilized in n-octylglucoside and subsequently incubated with protein A-

Sepharose beads, which had been preincubated with immunoglobulins from each of five individual patients and, five controls. Patient, but not control immunoglobulins were found to adsorb both the $H^+,K^+$-ATPase activity and the parietal cell antigen. Taken together, the results of study I showed that the major parietal cell antigen is $H^+,K^+$-ATPase, the proton pump of the stomach (70).

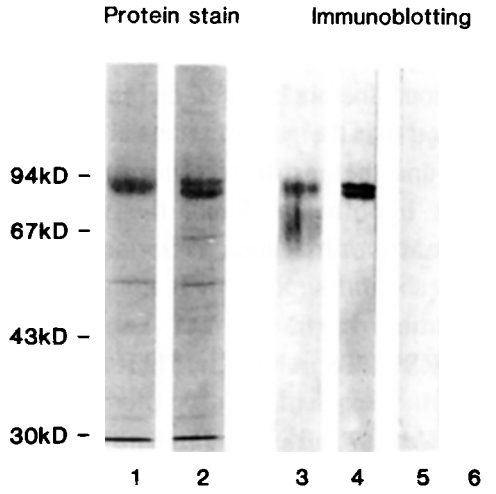


**Fig 3.** Analysis by SDS-PAGE and immunoblotting of vesicular membranes treated under nonreducing conditions (lanes 1, 3 and 5) after reduction and alkylation (lanes 2, 4 and 6). Protein staining by Coomassie Blue (lanes 1 and 2) and immunoblotting with a pool of 10 sera of patients with autoimmune gastritis (lanes 3 and 4) and sera of healthy controls (lanes 5 and 6).

**Comments:** On the basis of their finding of circulating antibodies blocking the gastrin receptor in subsets of patients with pernicious anemia, de Aizpurua et al (29) proposed the gastrin receptor to be a parietal cell antigen. The same group also described a non-glycosylated 65-70 kDa protein, present in parietal cell fractions from the dog and rat, and microsomes from the dog, rat and mouse, as the presumptive parietal cell autoantigen (35). As the gastrin receptor was later found in an elegant study by the same group (100) to be a 78 kDa protein, the nature of the non-glycosylated 65-70 kDa protein remained to be established. Our discovery of the $H^+,K^+$-ATPase as the major autoantigen (11a, 70) was subsequently confirmed by others. Goldkorn et al (46) found that sera containing PCA precipitated three antigens from a solubilized porcine gastric membrane preparation. These proteins migrated with apparent molecular masses

of 60-90, 92 and 100-120 kDa, respectively. Twenty-four out of 34 PCA-positive sera reacted only with the 60-90 kDa band on immunoblotting. The broad 100-120 band has since been found to be derived from the catalytic α subunit of $H^+,K^+$-ATPase (47, 68). The identities of the 65-75 kDa band observed in our experiments and the 60-90 kDa band described by Goldkorn et al were clarified in 1990 when several groups reported the existence of a second subunit, the β subunit of $H^+,K^+$-ATPase (13, 48, 106, 107, 124, 145). This protein is heavily glycosylated, colocalizes with the catalytic α subunit in tubulovesicular membranes and secretory canaliculi, and migrates as a 34 kDa protein on SDS-PAGE after deglycosylation. Toh et al, (145) showed that the β subunit could be coprecipitated by monoclonal antibodies to the catalytic 92 kDa moiety and vice versa. Goldkorn et al (46) found that antigenicity was abolished on pretreatment of the gastric membranes with N-glycanase, an enzyme which removed N-linked oligosaccharides, and also, in accordance with our own results, after reduction of the material. This would indicate that both the folding of the protein and the presence of carbohydrates are essential for antigenicity.

In the autoimmune gastritis induced by neonatal thymectomy in BALB/c mice, monoclonal antibodies directed against both 65-79 kDa and 92-120 kDa proteins, i.e. the same autoantigens as in human disease, have been obtained. The two types of monoclonal antibodies equally stained the membranes of parietal cell tubulovesicles and secretory canaliculi (99). On immunoblotting, 19 out of 20 sera from mice with autoimmune gastritis identified the catalytic α subunit. Six of these sera also recognized the β subunit, whereas one reacted with the β subunit only. In contrast, most sera, 71 %, of Finnish patients with pernicious anemia contained antibodies to the β chain, whereas only 20 % had antibodies to the catalytic α subunit, as assessed by an ELISA based on the purified subunits (95). We have found that in Western blots the majority of sera containing PCA react with both subunits, although with some sera the reactivity is confined to the broad, blurred 65-75 band. Also, in rare cases binding of antibodies to the gastric membrane fraction is detected by ELISA but not by Western blotting (not published), illustrating the heterogeneity of antigenic epitopes.

### *Effects of parietal cell antibodies on gastric acid formation and their deposition in the gastric mucosa*

In the second study, tubulovesicular gastric membranes with $H^+,K^+$-ATPase activity were incubated with the immunoglobulin fractions of 15 sera of patients with pernicious anemia, and with sera from ten healthy controls. The patient sera were all positive for PCA. The patient immunoglobulins were found to inhibit

the $H^+,K^+$-ATPase activity in relation to the titer of the parietal cell antibodies (Fig 4). Further, the activity of membrane-bound esterase remained unaltered, indicating that inhibition was confined to the $H^+,K^+$-ATPase. To examine the possible existence of gastrin receptor binding antibodies, a radioreceptor assay was established. The binding of $^{125}$I-gastrin to guinea pig gastric mucosal cells, containing 30 % parietal cells, was time- and dose-dependent. By Scatchard analysis one high affinity receptor with a binding constant of 1 x $10^9$ $M^{-1}$ was calculated. Immunoglobulins from 17 patient sera containing PCA did not interfere with gastrin binding (11b). The results of study II thus demonstrate direct inhibition of $H^+,K^+$-ATPase by parietal cell antibodies, but lack of interaction with the gastrin receptor. Absence of gastrin receptor blocking antibodies has recently also been shown by Smith et al (127).

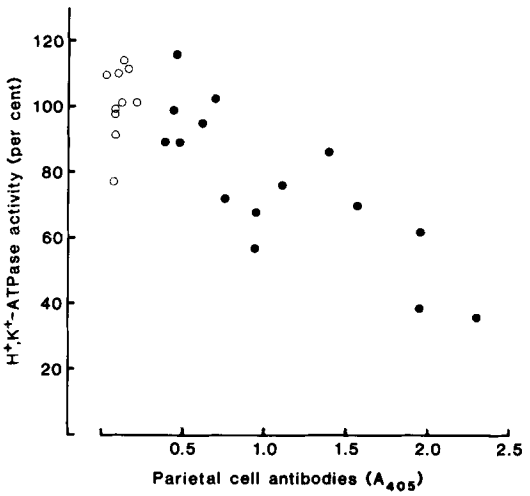


**Fig 4.** Effect of immunoglobulin preparations containing PCA on $H^+,K^+$-ATPase of vesicular membranes from porcine gastric mucosa. Then mean value of $H^+,K^+$-ATPase activity, when sera from 10 healthy individuals were used, was set as 100 %. $H^+,K^+$-adenosine triphosphatase was determined as the amount of $^{32}PO_4$ formed from [$^{32}$P]ATP. The degree of hydrolysis of [$^{32}$P]ATP amounted to 6.3 %- 18.6 % in the presence of the enzyme and about 1 % in the absence. Open circles = healthy controls, solid circles = patients with pernicious anemia.

In a separate study biopsy specimens from the gastric body mucosa were obtained from nine women, aged 27-44 years, all of whom had circulating PCA for at least 5 years. Diffuse mucosal infiltration by mononuclear cells was found in four subjects, and focal lymphocytic infiltrates in two. In the women with

diffuse infiltration, of whom one had intact gastric glands and three had various degrees of atrophy, all residual parietal cells were found to contain IgG. In one further woman with only mild, focal inflammation, IgG and to a lesser extent IgA were associated with the basolateral surfaces of some oxyntic glands. A parietal cell-specific location of the IgG was demonstrated by paired staining with a monoclonal antibody to $H^+,K^+$-ATPase. This suggests that in vivo the parietal cell antibodies have access to the antigen. Further, the presence of IgG in parietal cells at early stages of the disease could indicate that the autoantibodies have a pathogenetic role in the development of achylia and loss of parietal cells.

**Comments:** Our finding of a direct inhibitory effect of PCA on the proton pump (ATP-ase activity) is supported by the observation by Loveridge et al (87) of inhibitory effects of PCA on spontaneous and gastrin-stimulated acid secretion from strips of the bull frog gastric mucosa. To what extent PCA contributes to a reduction of HCl production in vivo has not yet been determined. Passive transfer of immunoglobulins from pernicious anemia patients to rats resulted in reduced secretion of gastric acid and atrophy of oxyntic glands (141). On the other hand, in the BALB/c mice model for autoimmune gastritis, transfer of spleen lymphocytes, but not sera, caused autoimmune gastritis in recipient mice (42). In the study of Tanaka and Glass the immunoglobulins were repeatedly injected over one month, but in the report by Fukuma et al (42) the doses and iterations of transferred sera are not given.

In the immunofluorescence test parietal cell antibodies appear to stain the cytoplasm of the parietal cells, and in our hands, an identital staining pattern is obtained with a monoclonal against $H^+,K^+$-ATPase (Fig 5). The finding of in situ deposits of IgG in intact parietal cells favors the idea of direct access of antibodies to the antigen, $H^+,K^+$-ATPase. The enzyme is normally situated on the apical cell membrane and in intracellular tubulovesicles, and not on the basolateral cell membrane, which is the membrane in contact with the circulation. In thyroid follicles from patients with autoimmune thyroiditis, IgG has been found in association with the apical cell membrane, and in some instances also on the basolateral membrane (77). It might be conjectured that upon chemical or microbiological damage to the gastric mucosa, antigens could be exposed or intercellular leakage of immunoglobulins take place, such that antibodies bind to antigen on the secretory membrane. Although not proven, the tissue-bound antibodies may not only be passive by-standers but might contribute to tissue damage by attracting Fc receptor-bearing mononuclear cells. The mechanism by which the parietal cells are altered to allow deposition of autoantibodies will be a subject of future studies.

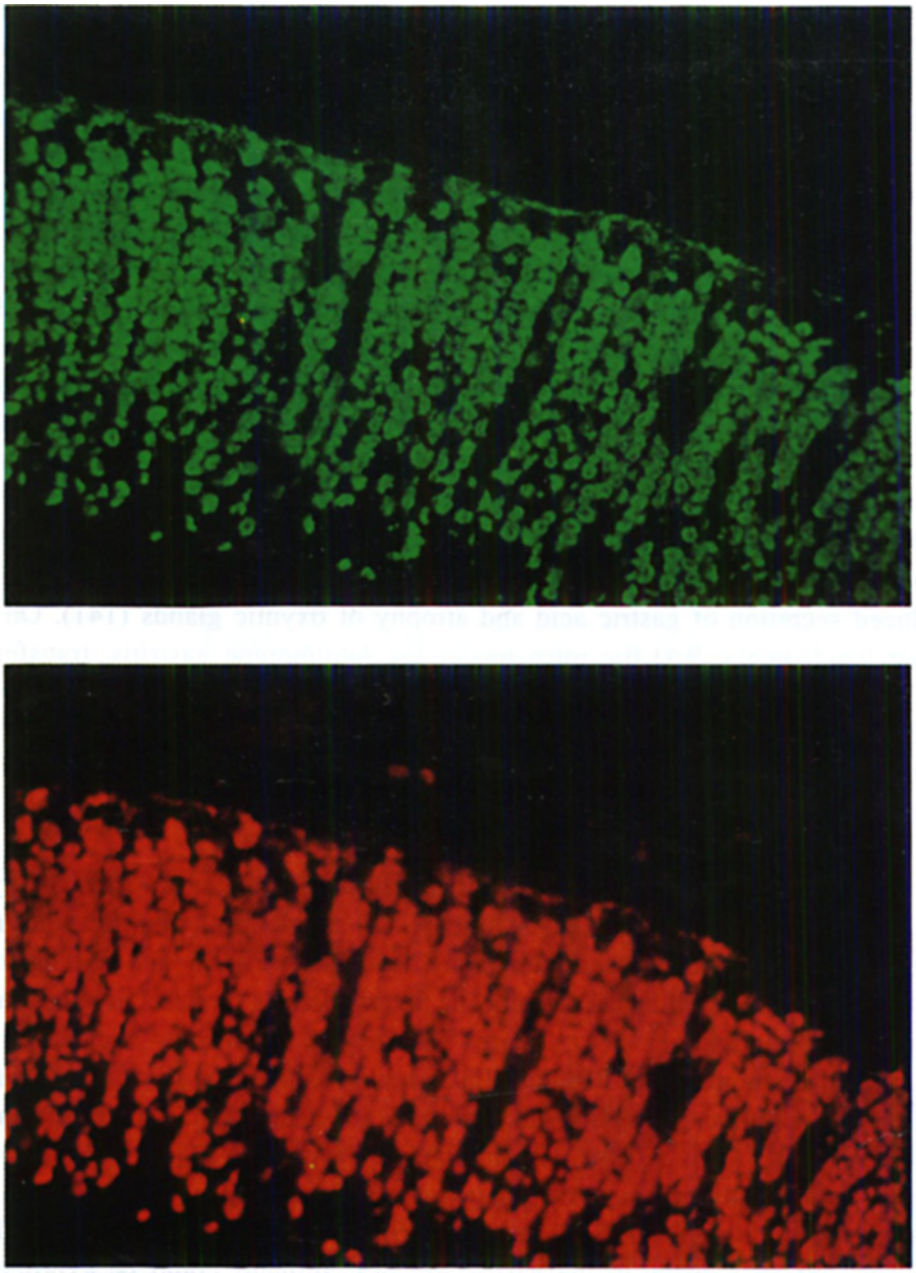

**Fig 5.** Cryostat sections of rat gastric mucosa incubated with serum containing parietal cell antibodies (upper, green). Binding was detected in the fluorescent microscope after addition of fluorescein-conjugated rabbit antibodies to human IgG. The presence of $H^+,K^+$-ATPase in the gastric mucosa was visualized after incubation with a murine monoclonal antibody to the enzyme and subsequent addition of rhodamine-conjugated rabbit anti-mouse immunoglobulin (lower, red). Note the identical staining pattern.

***$H^+,K^+$-ATPase antibodies in patients with autoimmune gastritis***

In study III we took advantage of a large material of patients with autoimmune gastritis, well characterized with respect to gastric mucosal function, and determined the presence of $H^+,K^+$-ATPase antibodies. The majority of the patients, 69 out of 86, also had pernicious anemia of various duration. Multiple biopsies from both the antrum and body mucosa were collected and the relative density of oxyntic glands was calculated. A correlation between serum pepsinogen A and the proportion of residual glands was found. Elevated antibody titers were noted in 93 % of the patients with pernicious anemia. Women had significantly higher titers than men. The antibody levels gradually decreased over decades and the titers correlated to pepsinogen A, but not to the gastrin levels. Nine of the 17 patients with simple atrophic gastritis had antibodies to $H^+,K^+$-ATPase and the titers were related to the severity of the mucosal gland destruction (11c).

**Comments:** In this study a high prevalence of antibodies to $H^+,K^+$-ATPase was observed in patients with pernicious anemia. This result is supported by the findings in previous investigations in which parietal cell antibody levels have been measured by immunofluorescence (25, 34, 45, 63, 147). We have previously compared the titer of antibodies in the ELISA with the result of the immunofluorescence test in routine use at our hospital, and found a good correlation (69). These data indicate that the majority of the immunoglobulins bound to parietal cells as observed by immunofluorescence tests are directed against the $H^+,K^+$-ATPase. If a further antigen exists, it must be of minor quantitative importance or coexist with $H^+,K^+$-ATPase. Whether the chief cells, which also are depleted in autoimmune gastritis, are the targets of autoimmune attacks or merely sensitive to locally produced cytokines is not known. However, since sera of patients bind to parietal cells and not to chief cells (Fig 5), B lymphocyte-mediated destruction seems less likely. A report of antibodies to pepsinogen by Mårdh and Song (101) awaits confirmation.

In longitudinal studies the presence of PCA has been found to be associated with development of severe atrophic gastritis (54). This is consistent with our finding of a higher prevalence of antibodies against $H^+,K^+$-ATPase in advanced compared with early stages of autoimmune gastritis. The ultimate loss of oxyntic glands in long-standing disease and the concomitant decline of the antibody titer suggests that the immune response is antigen driven. In autoimmune thyroid disease, TSH stimulation of the thyroid follicles results in higher levels of thyroid antibodies (65). To what extent alterations in the metabolic activity of the parietal cell might affect the intensity of the autoimmune process has not been elucidated.

***Postpartum gastritis***

During pregnancy and the postpartum period immunological changes occur that may have relevance for the course of autoimmune disorders (73). The postpartum period has a significant impact on the development of autoimmune thyroiditis (4, 64), and as both gastric autoantibodies and pernicious anemia are prevalent in patients with autoimmune thyroid diseases (61, 152), a study was made of the possible existence of a postpartum gastritis among women with postpartum thyroiditis. Antibodies to $H,^{+}K^{+}$-ATPase were found in 18 of 54 women (33 %), and in ten of them a two- to nine-fold elevation of the antibody titers was observed after delivery. The mean serum gastrin values in the early (<5 months) and late (>5 months) postpartum period in the three women with the most pronounced antibody increase postpartum were 17, 19, 28 and 33, 42, 40 pmol/l, respectively. At follow-up five years later the initially PCA-positive women still had elevated antibody levels. Hypergastrinemia and low serum pepsinogen A were found in four subjects; two of these had developed low vitamin $B_{12}$ accompanied by neurological symptoms and one was anemic. Gastric biosy specimens were obtained in nine cases (see above) and showed atrophic changes in three of them. In six cases the mucosa contained a mononuclear infiltrate dominated by T lymphoctes (CD3+) and macrophages (Leu-M3+) combined with an aberrant epithelial expression of HLA-DR. In the three women with the lowest antibody titer the gastric mucosa was normal. Further, it was discovered that IgG was selectively localized to the parietal cells (discussed above).

**Comments:** In this study evidence was found of a postpartum gastritis syndrome occurring in a fraction of women with autoimmune thyroiditis. Although serum gastrins in the three women with the most prominent antibody titer elevation (gastrins were not consecutively determined after delivery in the others) were higher in the late than in the early postpartum period, the levels did not reach those usually found in achylia. Of more clinical relevance is the finding of advanced gastritis in 18 % of the antibody-positive women, since this is known to carry an increased risk of developing vitamin $B_{12}$ malabsorption. Indeed, two of the women already had low vitamin $B_{12}$ levels with neurological symptoms at the follow-up. A similar clinical presentation has recently attracted attention (14, 83). In one of these women iron deficiency anemia, another sequela of atrophic gastritis (71), was found. Since the overall prevalence of pernicious anemia in Sweden is about 0.2 % (10) and since it usually affects elderly people, the relatively high incidence of low serum cobalamin in women with postpartum thyroiditis may suggest that such women should be followed up at regular intervals.

***A role of autoimmunity in peptic ulcer disease?***

Peptic ulcer is a multifactorial disease resulting from an imbalance between aggressive factors such as gastric acid and proteases, and protective mucosal defense mechanisms. *Helicobacter pylori* infection has recently been linked to recurrence of ulcers after treatment with acid-reducing drugs (23, 92), and the possibility of a causal relationship is under current debate. Cigarette smoking has been identified as a risk factor for impaired healing as well as recurrence (129). Hypersecretion of gastric acid has been shown in about one third of patients (131). An immunologic origin of ulcer disease has been proposed (112). In 1982 Dobi and Lenkey reported that immunoglobulin preparations from 25 out of 51 patients with severe ulcer disease stimulated gastric acid secretion when injected into rats (33). De Lazarri and co-workers (30) found antibodies stimulating cyclic AMP production in a gastric mucosal cell preparation, and suggested the presence of $H_2$-receptor stimulatory antibodies in subgroups of patients with peptic ulcer disease. This proposal was examined in the present study.

An assay for cAMP production of isolated porcine gastric mucosal cells was developed. The release of cAMP into medium was measured with a radioimmunoassay. The amount released into the medium increased during 4-hour incubations and was histamine responsive. An approximately 20-fold increase was found with histamine at a concentration of $10^{-4}$ M. Sera from healthy blood donors showed approximately 20 % inhibition of cAMP production, whereas the IgG fractions had weaker non-specific effects. No stimulation was found when sera and IgG fractions from 57 patients with ulcer disease, of whom 32 were classified as poor responders to treatment, were tested (11d).

**Comments:** Histamine, which plays an essential role in gastric acid production, mainly elicits its effects via the formation of cAMP. In cell separation studies, histamine-stimulated cAMP production seemed to be restricted to the parietal cells (132). In contrast to de Lazarri and associates, who used guinea pig gastric mucosal cells, we found no stimulatory effect of sera or immunoglobulins from patients. With reference to the severity of the disease, the patients of the two studies were comparable. Our test system, based on porcine gastric mucosa cells, was superior with respect to the response to histamine (20-fold compared with five-fold). In organ-specific autoimmune diseases with production of antibodies, tissue- but not species-specificity is the usual finding (158). The above mentioned observation by Dobi and Lenkey has not been confirmed and remains unexplained. The immunoglobulin fractions of their patient sera stained parietal

cells in an immunofluorescence test. However, we and others (148) have not found an increased incidence of PCA in patients with duodenal ulcer. Thus, we consider gastric stimulatory antibodies an unlikely cause of severe ulcer disease.

## GENERAL SUMMARY

$H^+,K^+$-ATPase, the acid-producing enzyme of the stomach, was identified as the major parietal cell antigen in autoimmune gastritis.

Parietal cell antibodies were found to inhibit the activity of $H^+,K^+$-ATPase in vitro but not to interfere with the binding of gastrin in a radioreceptor assay. The magnitude of enzyme inhibition was related to the antibody titer, suggesting that patient antibodies recognize a limited number of antigenic epitopes. The observed inhibition of the antibodies in vitro possibly contributes to reduce gastric acid formation in patients with autoimmune gastritis.

A high prevalence of antibodies to $H^+,K^+$-ATPase was observed in patients with pernicious anemia. The titer was related to the duration of the disease, and the residual parietal cell mass indicating that the autoimmune response is antigen driven.

Evidence of postpartum activation of autoimmune gastritis was obtained in a study of women with parietal cell antibodies and coexisting postpartum autoimmune thyroiditis. At a follow-up investigation, such women were found to constitute a group with an increased risk of developing malabsorption of vitamin $B_{12}$ at an early age.

Gastric biopsy specimens from patients with early atrophic gastritis showed lymphocytic infiltrates, aberrant expression of HLA-D molecules and deposition of antibodies in the parietal cells. The presence of antibodies within parietal cells of intact oxyntic glands suggests that the antibodies are pathogenetically involved in the destruction and ultimate loss of parietal cells.

Antibodies stimulating the $H_2$ receptors of parietal cells were not found in patients with severe ulcer disease. The findings contradict a recent hypothesis of an autoimmune origin of peptic ulcers in patients poorly responsive to treatment with anti-secretory drugs.

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Adress for reprints:
Pia Burman
Department of Internal Medicine
University Hospital
S-751 85 Uppsala
Sweden