

Review Article

*Mechanisms of Disease*FRANKLIN H. EPSTEIN, M.D., *Editor*

PERNICIOUS ANEMIA

BAN-HOCK TOH, M.B., B.S., D.Sc.,
IAN R. VAN DRIEL, Ph.D., AND PAUL A. GLEESON, Ph.D.

PERNICIOUS anemia is the most common cause of vitamin B₁₂ deficiency. Vitamin B₁₂ deficiency has many causes; the term "pernicious anemia" applies only to the condition associated with chronic atrophic gastritis. A recent population survey revealed that 1.9 percent of persons more than 60 years old have undiagnosed pernicious anemia.¹ Earlier studies suggested that pernicious anemia is restricted to Northern Europeans.² However, subsequent studies have reported the disease in black and Latin-American subjects, with an earlier age of onset in black women.^{1,3} Although the disease is silent until the end stage, the underlying gastric lesion can be predicted many years before anemia develops.

Pernicious anemia was first described by Thomas Addison in 1849.⁴ The anemia was linked to the stomach by Austin Flint in 1860 and named pernicious anemia soon thereafter. Successful treatment of the anemia with cooked liver suggested that it was caused by the lack of an extrinsic factor that was found in liver (later identified as vitamin B₁₂) and an intrinsic factor in gastric juice.⁴ Although pernicious when first discovered, the disease is now controlled by treatment with vitamin B₁₂. The discovery of a serum inhibitor of intrinsic factor⁵ (later found to be an autoantibody to intrinsic factor) and of autoantibodies to parietal cells⁶ laid the foundation for the immunologic explanation of the underlying gastritis that causes pernicious anemia.

From the Department of Pathology and Immunology, Monash University Medical School, Prahran, Victoria 3181, Australia, where reprint requests should be addressed to Dr. Toh.
©1997, Massachusetts Medical Society.

GASTRIC PATHOLOGICAL FINDINGS

Gross Pathological Findings

There are three regions of the stomach: the fundus and the body, both of which contain acid-secreting gastric parietal cells and pepsinogen-secreting zymogenic cells, and the antrum, which contains gastrin-producing cells. Chronic atrophic gastritis is recognized macroscopically by the loss of gastric mucosal folds and thinning of the gastric mucosa. It can be classified into two types according to whether or not the lesion affects the gastric antrum (Table 1).⁷ Type A (autoimmune) gastritis involves the fundus and body of the stomach and spares the antrum, whereas type B (nonautoimmune) gastritis involves the antrum as well as the fundus and body. Type A gastritis is associated with pernicious anemia, autoantibodies to gastric parietal cells and to intrinsic factor, achlorhydria, low serum pepsinogen I concentrations, and high serum gastrin concentrations, the latter resulting from hyperplasia of gastrin-producing cells. Type B gastritis is usually associated with *Helicobacter pylori* infection and low serum gastrin concentrations, because of destruction of the gastrin-producing cells associated with antral gastritis.⁸

Histopathological Findings

Gastric-biopsy specimens from patients with pernicious anemia show a mononuclear cellular infiltrate in the submucosa extending into the lamina propria between the gastric glands (Fig. 1). The cellular infiltrate includes plasma cells, T cells, and a large non-T-cell population (probably B cells).⁹ The infiltrating plasma cells contain autoantibodies to the parietal-cell antigen and to intrinsic factor.¹⁰ Extension of the cellular infiltrate into the mucosa is accompanied by degenerative changes in parietal

TABLE 1. TYPES OF CHRONIC GASTRITIS.

Type A gastritis
Antrum spared
Antibodies to parietal cells and intrinsic factor
Low serum pepsinogen I concentrations
Achlorhydria
Hypergastrinemia and gastric carcinoids
Vitamin B ₁₂ -deficient megaloblastosis
Type B gastritis
Antrum involved
<i>Helicobacter pylori</i> infection
Hypogastrinemia

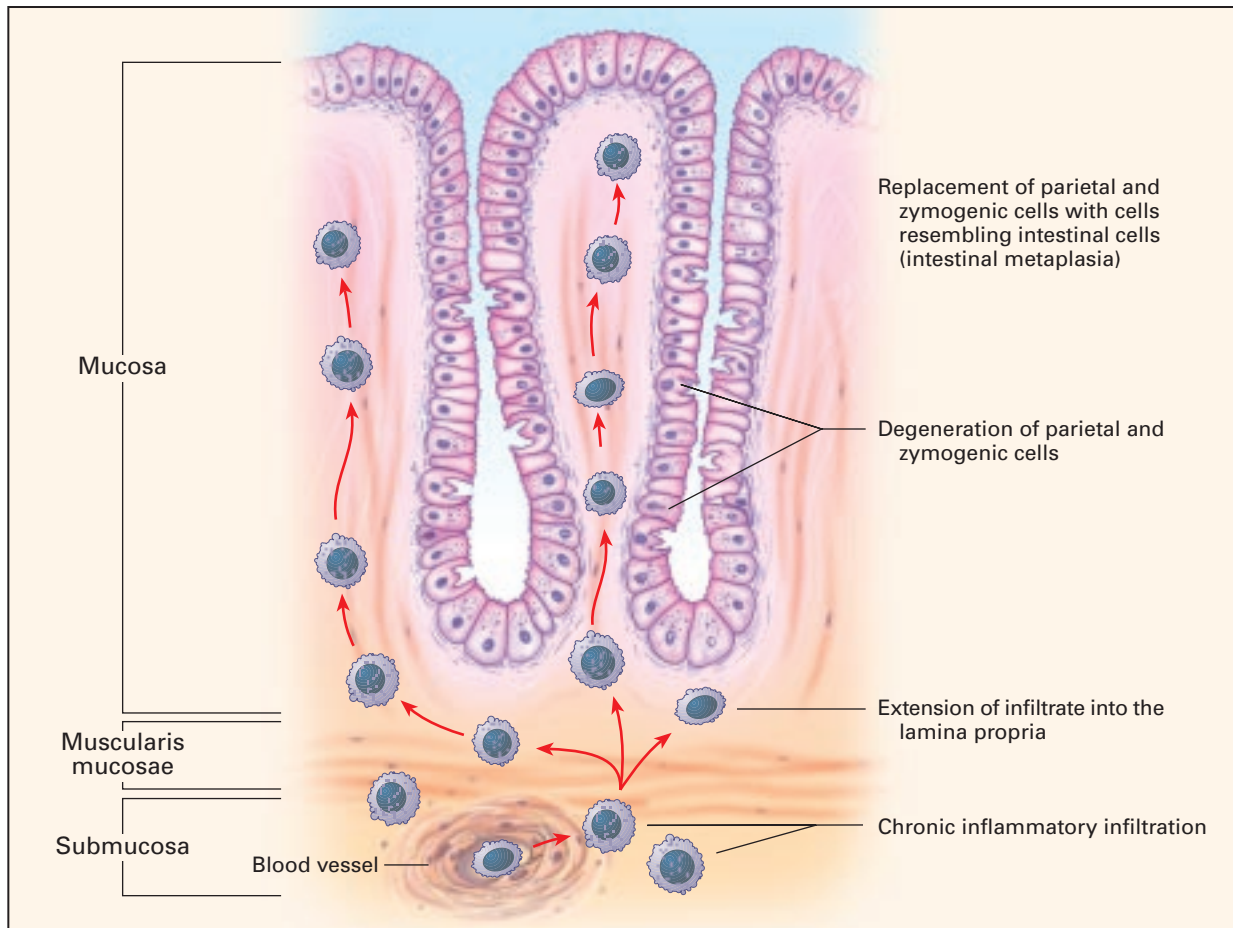


Figure 1. The Gastric Lesion of Pernicious Anemia.

The early lesion is characterized by chronic infiltration of the gastric submucosa with inflammatory cells. Extension of the chronic inflammatory infiltrate into the lamina propria is associated with degeneration of gastric parietal and zymogenic cells. The advanced lesion is characterized by loss of parietal and zymogenic cells and replacement by cells resembling those of the intestinal mucosa (intestinal metaplasia).

cells and zymogenic cells. In the fully established lesion, there is marked reduction in the number of gastric glands, and the parietal cells and zymogenic cells disappear and are replaced by mucus-containing cells (intestinal metaplasia).

NATURAL HISTORY

The progression of type A chronic atrophic gastritis to gastric atrophy and clinical anemia is likely to span 20 to 30 years. The presence of gastric parietal-cell antibodies in the serum is predictive of the presence of autoimmune gastritis.¹¹ The pathologic lesion and the anemia can be reversed by treatment with corticosteroids¹² or azathioprine.¹³ These observations suggest that precursor cells present in the stomach can differentiate into parietal and zymogenic cells if further autoimmune destruction is controlled. This suggestion is supported by studies of mice with autoimmune gastritis.

IMMUNOPATHOGENESIS OF GASTRITIS

Gastric Parietal-Cell $H^+/K^+-ATPase$

The pathologic process associated with type A gastritis appears to be directed toward the gastric parietal cells. The pathologic lesion is restricted to the parietal-cell-containing fundus and body regions of the stomach. Parietal cells are lost from the gastric mucosa, and autoantibodies to parietal cells and to their secretory product, intrinsic factor, are present in the serum and in gastric juice.

A major breakthrough in our understanding of the pathogenesis of type A gastritis was the demonstration that gastric $H^+/K^+-ATPase$ is the antigen recognized by parietal-cell autoantibodies.¹⁴⁻¹⁸ This ATPase belongs to a family of electroneutral P-type ATPases that includes $Na^+/K^+-ATPase$ and $Ca^{2+}-ATPase$.¹⁹ These enzymes have a highly conserved catalytic (α) subunit that is phosphorylated during

Figure 2. Gastric Parietal-Cell H^+/K^+ -ATPase as the Molecular Target in Autoimmune Gastritis Associated with Pernicious Anemia.

The top panel represents a gastric gland, showing the location of parietal cells in relation to zymogenic cells, immature cells, and surface mucous cells. The middle panel represents a stimulated gastric parietal cell, showing the lining membrane of the secretory canaliculus on which gastric H^+/K^+ -ATPase is located. The bottom panel represents the catalytic α and glycoprotein β subunits of gastric H^+/K^+ -ATPase, showing their orientation in the lining membrane of the secretory canaliculus of the parietal cell. N denotes the N-terminal of protein, and C the C-terminal of protein.

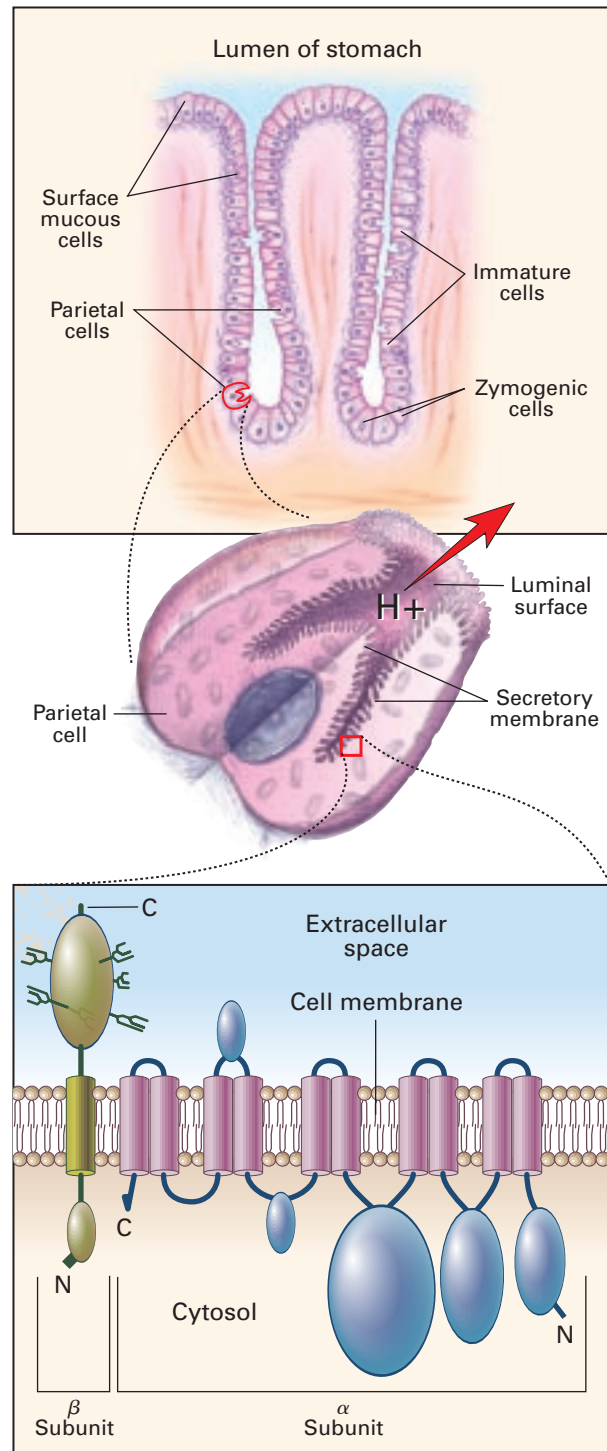
reaction cycles. Gastric H^+/K^+ -ATPase is responsible for secretion of hydrogen ions by parietal cells in exchange for potassium ions (Fig. 2). This enzyme is the major protein of the membrane lining the secretory canaliculi of parietal cells.²⁰⁻²² Autoantibodies to parietal cells bind to both the 100-kd catalytic (α) subunit and the 60-to-90-kd glycoprotein (β) subunit of gastric H^+/K^+ -ATPase.¹⁷

Although parietal-cell autoantibodies can fix complement²³ and lyse parietal cells in vitro,²⁴ it is unlikely that these autoantibodies are pathogenic in vivo, because gastric H^+/K^+ -ATPase is not accessible to circulating antibodies. The importance, if any, of an early observation that passive transfer of parietal-cell autoantibodies to rats resulted in reduction in parietal-cell mass without an inflammatory response is therefore uncertain.²⁵ A report describing autoantibodies that bind to the gastrin receptor²⁶ was not confirmed.²⁷ The results of studies showing reactivity of parietal-cell autoantibodies with the surface membranes of parietal cells in vitro^{28,29} may be explained by the loss of cell polarity after cellular dissociation. Gastric H^+/K^+ -ATPase appears to be the only parietal-cell antigen recognized by parietal-cell autoantibodies, because immunoblotting and immunoprecipitation experiments show reactivity only with the two subunits of this ATPase.¹⁴⁻¹⁸

Murine Models of Autoimmune Gastritis

The identification of gastric H^+/K^+ -ATPase as the target of parietal-cell autoantibodies raises the question of the role of the ATPase in the immunopathogenesis of the gastric lesion. Although this question has not been answered for pernicious anemia, studies in mice suggest that the lesion of autoimmune gastritis is initiated by CD4 T cells that recognize the β subunit of gastric H^+/K^+ -ATPase.

Organ-specific autoimmune disease, including gastritis, develops in susceptible strains of mice after neonatal thymectomy (Fig. 3, upper panel).³⁰⁻³² Gastritis also develops in neonatal mice treated with cyclosporine³³ and in adult mice after thymectomy combined with irradiation,³⁴ cyclophosphamide treat-



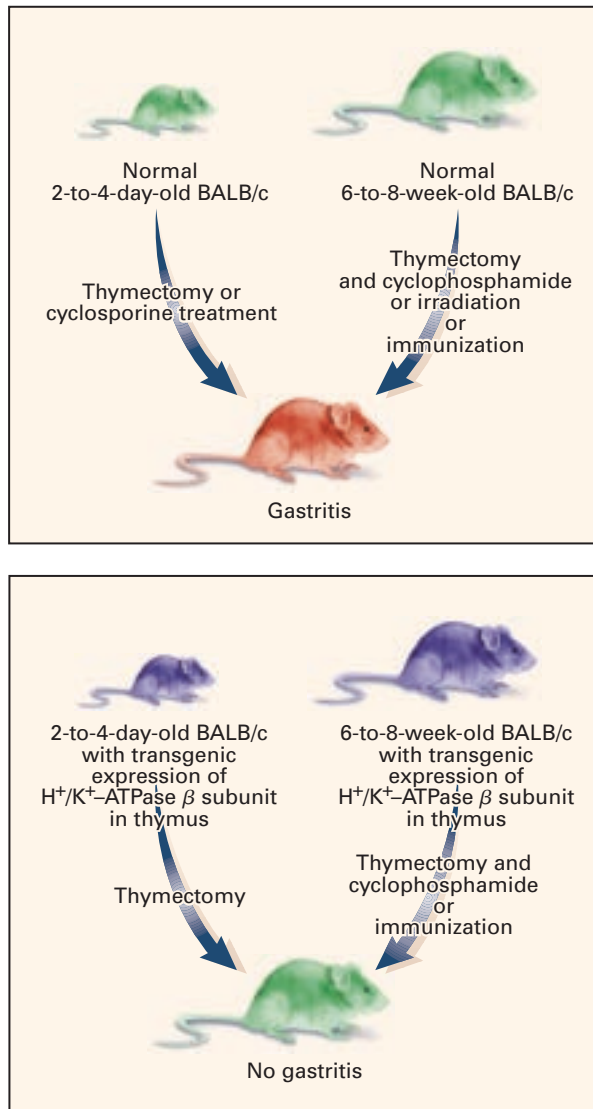


Figure 3. Experimental Autoimmune Gastritis in Mice.

Thymectomy or cyclosporine treatment of 2-to-4-day-old BALB/c mice, thymectomy of adult mice combined with cyclophosphamide treatment or irradiation, or immunization of adult mice with the gastric H^+/K^+ -ATPase results in autoimmune gastritis (upper panel).

Transgenic expression of the β subunit of the gastric H^+/K^+ -ATPase in the thymus of BALB/c mice prevents the development of gastritis induced by neonatal thymectomy, adult thymectomy combined with cyclophosphamide treatment, or immunization with the gastric H^+/K^+ -ATPase (lower panel).

ment, or immunization with murine gastric H^+/K^+ -ATPase.³⁵ This gastritis, like that of pernicious anemia, is characterized by submucosal infiltration of mononuclear cells extending into the lamina propria between the gastric glands, with loss of parietal and zymogenic cells. The mononuclear cells in early lesions are predominantly macrophages and CD4

T cells producing a mixture of Th1-type and Th2-type cytokines.³⁶ CD4 T cells that react with H^+/K^+ -ATPase are present in regional gastric lymph nodes of these mice.³⁷ Mice with gastritis also have serum autoantibodies to gastric H^+/K^+ -ATPase.³⁸ CD4 T cells appear to be important in the pathogenesis of the gastritis, because transfer of these cells into naive mice results in gastritis and serum autoantibodies to gastric H^+/K^+ -ATPase. Autoantibodies and CD8 T cells do not appear to have a role in the genesis of gastritis.

Transgenic expression of the β subunit of gastric H^+/K^+ -ATPase in the thymus prevents gastritis induced by neonatal thymectomy,³⁹ by adult thymectomy combined with cyclophosphamide treatment,³⁵ or by immunization with murine gastric H^+/K^+ -ATPase (Fig. 3, lower panel).⁴⁰ These observations suggest that the pathogenic T cells have been rendered tolerant after encountering the β subunit in the thymus. The α subunit, which is present in the normal thymus, does not appear to have a role in the initiation of gastritis⁴⁰ but may have a role in its perpetuation.⁴¹ A single injection of neutralizing anti-interferon- γ antibody prevents the development of gastritis, implicating this Th1-type cytokine in its genesis.⁴² Taken together, these observations suggest that interferon- γ -secreting Th1-type CD4 T cells are important in the pathogenesis of murine autoimmune gastritis (Fig. 4). Whether this is also the case for the gastritis of pernicious anemia in humans is not known. The gastric environment appears to be important for the genesis of the lesion, because transgenic expression of the β subunit of H^+/K^+ -ATPase in pancreatic islets does not induce a destructive insulinitis after neonatal thymectomy.⁴³

Tolerance of and Autoimmunity to Gastric H^+/K^+ -ATPase

Murine autoimmune gastritis occurs only when pathogenic T cells are transferred to immunocompromised mice. This observation, together with the induction of gastritis by thymectomy, immunosuppressive drugs, and irradiation, suggests that pathogenic T cells expand only in a lymphopenic host.³² Expansion of these pathogenic T cells can be prevented by transfer of normal adult CD4 T cells. The pathogenic CD4 T cells are probably "resting" T cells, because they do not express the T-cell-activation marker CD25, whereas disease-preventing CD4 T cells express CD25.⁴⁴ Furthermore, gastritis can be induced in mice after the administration of antibodies to CD25.⁴⁵ The mechanisms that prevent the activation and expansion of "resting" pathogenic T cells and their homing to the stomach in normal subjects are not known. The simplest explanation is competition for space by CD4 T cells within defined lymphoid compartments, a process that appears to be under as yet undefined homeostatic control.⁴⁶

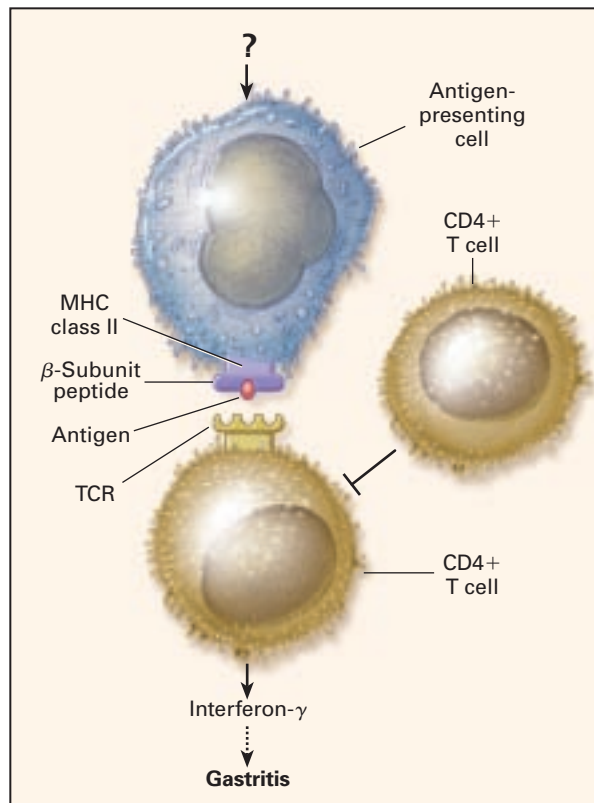


Figure 4. The Development of Autoimmune Gastritis.

CD4 Th1 T cells are activated by binding of their T-cell antigen receptors (TCR) with a complex of a peptide of the β subunit of the gastric $H^+/K^+-ATPase$ and a major histocompatibility complex (MHC) class II molecule on antigen-presenting cells. The mechanism of activation of the antigen-presenting cells and the pathway by which pathogenic T cells mediate gastritis are not known. Other CD4 T cells may prevent expansion of pathogenic CD4 T cells.

Destruction of Zymogenic Cells

In both human and murine autoimmune gastritis, zymogenic cells are lost together with parietal cells from the gastric mucosa. There is no evidence of an autoimmune reaction directed toward zymogenic cells. Therefore, the loss of these cells is probably secondary to the primary autoimmune reaction directed toward parietal-cell $H^+/K^+-ATPase$. The induction of autoimmune gastritis by direct immunization with gastric $H^+/K^+-ATPase$ in Freund's adjuvant is also characterized by loss not only of parietal cells but also of zymogenic cells.⁴⁷

MECHANISMS OF VITAMIN B_{12} MALABSORPTION

Intrinsic factor is a 60-kd glycoprotein produced by gastric parietal cells that avidly binds dietary vita-

min B_{12} . The vitamin B_{12} -intrinsic factor complex is carried to the terminal ileum, where it is absorbed after binding to intrinsic-factor receptors on the luminal membranes of ileal cells.⁴⁸ Malabsorption of vitamin B_{12} in patients with pernicious anemia is due to intrinsic-factor deficiency. Two mechanisms are responsible. First, the progressive destruction and eventual loss of parietal cells from the gastric mucosa lead to failure of intrinsic-factor production. Indeed, the severity of the gastric lesion correlates with the degree of impaired secretion of intrinsic factor and the reduction in vitamin B_{12} absorption. Second, blocking autoantibodies present in the gastric juice can bind to the vitamin B_{12} -binding site of intrinsic factor, thereby preventing the formation of the vitamin B_{12} -intrinsic factor complex. Vitamin B_{12} is required for DNA synthesis. Therefore, the major organs affected by vitamin B_{12} deficiency are those in which cell turnover is rapid, such as the bone marrow and the gastrointestinal tract.

PREDISPOSING GENETIC FACTORS

A genetic predisposition to pernicious anemia is suggested by the clustering of the disease and of gastric autoantibodies in families, and by the association of the disease and gastric autoantibodies with the autoimmune endocrinopathies. There are reports of a number of white families with a high frequency of pernicious anemia over several generations. About 20 percent of the relatives of patients with pernicious anemia have pernicious anemia. These relatives, especially first-degree female relatives, also have a higher frequency of gastric autoantibodies than normal subjects. Concordance with respect to pernicious anemia has been observed in 12 sets of monozygotic twins, implicating a strong genetic predisposition to development of the disease.⁴⁹ In contrast to some other autoimmune diseases, there is little evidence of an association between pernicious anemia and particular molecules of the major histocompatibility complex.⁵⁰

ASSOCIATION WITH OTHER AUTOIMMUNE DISEASES

Pernicious anemia may be associated with autoimmune endocrinopathies and antireceptor autoimmune diseases. These diseases include chronic autoimmune thyroiditis (Hashimoto's thyroiditis), insulin-dependent diabetes mellitus, Addison's disease, primary ovarian failure, primary hypoparathyroidism, Graves' disease, vitiligo, myasthenia gravis, and the Lambert-Eaton syndrome.⁵¹

CLINICAL PRESENTATION

Anemia

Animal products are the primary dietary source of vitamin B_{12} . The recommended daily dietary allowance of the vitamin is 2 μg .⁵² The average Western

diet provides an excess of the vitamin (3 to 9 μg per day), which is stored mainly in the liver.

The onset and progression of pernicious anemia are slow. The median age at diagnosis is 60 years. Slightly more women than men are affected. The usual presentation is with symptoms of anemia; asymptomatic patients can be identified by routine hematologic investigations.

Gastrointestinal Manifestations

Vitamin B₁₂ deficiency results in several abnormalities of the digestive tract. The tongue is usually smooth and beefy red because of atrophic glossitis. Megaloblastosis of the epithelial cells of the small intestine may result in diarrhea and malabsorption.

Neurologic Complications

Vitamin B₁₂ deficiency may cause peripheral neuropathy and lesions in the posterior and lateral columns of the spinal cord (subacute combined degeneration) and in the cerebrum. These lesions progress from demyelination to axonal degeneration and eventual neuronal death. These are serious complications, because they may not be reversed after replacement therapy with vitamin B₁₂. The most frequent manifestations of peripheral neuropathy are paresthesias and numbness. The manifestations of a lesion in the spinal cord are a mixture of signs of a posterior column lesion (loss of vibration and position sense, and sensory ataxia with positive Romberg's sign) and those of a lateral column lesion (limb weakness, spasticity, and extensor plantar responses). Cerebral manifestations range from mild personality defects and memory loss to frank psychosis ("megaloblastic madness").

Gastric Complications

Intestinal metaplasia is a risk factor for adenocarcinoma. Achlorhydria and bacterial overgrowth may also lead to the formation of carcinogenic nitrosoamines. Population-based studies have revealed an excess risk of gastric carcinoma as well as gastric carcinoid tumors in patients with pernicious anemia. The gastric carcinoid tumors are probably due to hypergastrinemia. The evolution of these endocrine cells from hyperplasia to neoplasia has been attributed to the trophic action of gastrin. In a recent population-based cohort study in Sweden, the risk of gastric carcinoma was increased 3 times and that of gastric carcinoid tumors 13 times in patients with pernicious anemia.⁵³ The prevalence of gastric carcinoma in patients with pernicious anemia is 1 to 3 percent, and 2 percent of patients with gastric carcinoma have pernicious anemia. One study suggested that regular endoscopic surveillance is warranted in patients with pernicious anemia.⁵⁴

Pernicious Anemia and Immunodeficiency

Pernicious anemia associated with common variable immunodeficiency and low serum immuno-

globulin concentrations or with selective IgA deficiency should be distinguished from classic pernicious anemia. It occurs in younger patients and has the features of a type B gastritis.⁵⁵

Childhood Pernicious Anemia

Childhood pernicious anemia is also not associated with chronic atrophic gastritis or achlorhydria but is the result of a genetically determined failure to secrete intrinsic factor⁵⁶ or the secretion of a defective intrinsic factor.⁵⁷

LABORATORY DIAGNOSIS

Hematologic Studies

In established megaloblastic anemia, examination of the peripheral blood reveals macrocytosis with hypersegmented polymorphonuclear leukocytes (Fig. 5), anemia, leukopenia, and thrombocytopenia or pancytopenia. Examination of bone marrow reveals megaloblasts and large myeloid precursors ("giant metamyelocytes"). Examination of the marrow is not indicated if the diagnosis is unequivocal. Vitamin B₁₂ deficiency as the cause of megaloblastic anemia is established by a low serum vitamin B₁₂ concentration and normal serum folate concentration.

A Schilling test will confirm that the vitamin B₁₂ deficiency is the result of intestinal malabsorption due to intrinsic-factor deficiency. In patients with pernicious anemia, urinary excretion of orally administered vitamin B₁₂ is low, and it increases if vitamin B₁₂ is administered with intrinsic factor. A simpler test is measurement of serum holotranscobalamin II, the circulating protein that delivers vitamin B₁₂ to cells. In patients with vitamin B₁₂ deficiency, serum concentrations of holotranscobalamin II fall before those of vitamin B₁₂.⁵²

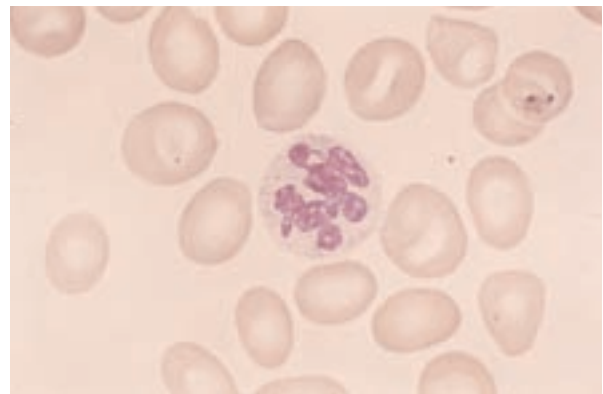


Figure 5. Peripheral-Blood Film Showing Macrocytic Red Cells and a Hypersegmented Polymorphonuclear Leukocyte from a Patient with Pernicious Anemia.

Photomicrograph courtesy of Dr. Alison Street, Haematology Unit, Pathology Services, Alfred Hospital, Melbourne, Australia.

Serologic Studies

Serum antibodies to gastric parietal cells can be detected by indirect immunofluorescence with unfixed, air-dried, frozen sections of mouse stomach in which the antibodies stain parietal cells. Mouse stomachs are preferable to rat stomachs because the latter may give false positive heterophile reactions.⁵⁸ These autoantibodies are found in about 90 percent of patients with pernicious anemia but also in about 30 percent of nonanemic first-degree relatives of patients with pernicious anemia and in patients with autoimmune endocrinopathies. In normal subjects there is an age-related increase in the prevalence of parietal-cell autoantibodies, from 2.5 percent in the third decade to 9.6 percent in the eighth decade.⁵⁹ The explanations for the seronegative results in 10 percent of patients with pernicious anemia include faulty diagnosis, complete binding of antibody to antigen so that none is circulating at the time of measurement, disappearance of antibody because of disappearance of the antigen, or failure of production of the antibody.

Two types of autoantibodies to intrinsic factor have been described.⁶⁰ Type I autoantibodies block the binding of vitamin B₁₂ to intrinsic factor. They are demonstrable in the serum of about 70 percent of patients with pernicious anemia. Type II autoantibodies bind to a site remote from the vitamin B₁₂-binding site, are found in the serum of about 35 to 40 percent of patients, and rarely occur in the absence of the first type of antibody. However, the use of a sensitive enzyme-linked immunosorbent assay for the detection of both autoantibodies has shown that type II autoantibodies appear to be more common than previously reported.⁶¹ Both types of autoantibodies can be detected more frequently in gastric juice than in the serum. The demonstration of circulating intrinsic-factor autoantibodies is almost diagnostic of type A gastritis and pernicious anemia.

Gastric Biopsy, Achlorhydria, and Serum Pepsinogen Concentrations

The presence of type A chronic atrophic gastritis can be confirmed by gastric biopsy. Total (pentagastrin-resistant) achlorhydria, the direct result of the loss of gastric parietal cells, is diagnostic of pernicious anemia because it is the only gastric lesion that results in total achlorhydria. Hypergastrinemia is the result of sparing of the antrum and stimulation of the gastrin-producing G cells by achlorhydria. A low serum pepsinogen I concentration is the result of the destruction of the chief cells.

TREATMENT

The standard treatment is regular monthly intramuscular injections of at least 100 μ g of vitamin B₁₂ to correct the vitamin deficiency.⁵² This treatment corrects the anemia and may correct the neurologic

complications if given soon after their onset. It has been suggested that elderly patients with gastric atrophy should take tablets containing 25 μ g to 1 mg of vitamin B₁₂ daily to prevent vitamin B₁₂ deficiency.⁵² This recommendation is based on the observation that about 1 percent of vitamin B₁₂ is absorbed by mass action in the absence of intrinsic factor.

CONCLUSIONS

Pernicious anemia is the end stage of type A chronic atrophic (autoimmune) gastritis. The gastritis results in the loss of parietal cells in the fundus and body of the stomach. The loss of these cells is associated with the failure of intrinsic-factor production and results in vitamin B₁₂ deficiency and megaloblastic anemia. An autoimmune basis for the gastritis is supported by the presence of mononuclear-cell infiltration into the gastric mucosa with loss of parietal and zymogenic cells, autoantibodies to parietal cells and intrinsic factor, regeneration of parietal and zymogenic cells after therapy with corticosteroids or immunosuppressive drugs, familial predisposition, and association with autoimmune endocrinopathies and antireceptor autoimmune diseases.

The identification of gastric H⁺/K⁺-ATPase as the target of parietal-cell autoantibodies was a major breakthrough in our understanding of the molecular and immunologic basis of autoimmune gastritis. The immunologic mechanisms that allow the initiation and progression of the T-cell response to this enzyme, leading to autoimmune gastritis, remain to be established.

Supported by grants from the National Health and Medical Research Council of Australia.

REFERENCES

1. Carmel R. Prevalence of undiagnosed pernicious anemia in the elderly. *Arch Intern Med* 1996;156:1097-100.
2. Nutritional folate deficiency. In: Chanarin I. The megaloblastic anaemias. 2nd ed. Oxford, England: Blackwell Scientific, 1979:457-65.
3. Carmel R, Johnson CS. Racial patterns in pernicious anemia: early age at onset and increased frequency of intrinsic-factor antibody in black women. *N Engl J Med* 1978;298:647-50.
4. Castle WB. Development of knowledge concerning the gastric intrinsic factor and its relation to pernicious anemia. *N Engl J Med* 1953;249:603-14.
5. Taylor KB. Inhibition of intrinsic factor by pernicious anaemia sera. *Lancet* 1959;2:106-8.
6. Taylor KB, Roitt IM, Doniach D, Couchman KG, Shapland C. Autoimmune phenomena in pernicious anaemia: gastric antibodies. *BMJ* 1962;2:1347-52.
7. Strickland RG, Mackay IR. A reappraisal of the nature and significance of chronic atrophic gastritis. *Am J Dig Dis* 1973;18:426-40.
8. Fong TL, Dooley CP, Dehesa M, et al. Helicobacter pylori infection in pernicious anemia: a prospective controlled study. *Gastroenterology* 1991;100:328-32.
9. Kaye MD, Whorwell PJ, Wright R. Gastric mucosal lymphocyte subpopulations in pernicious anemia and in normal stomach. *Clin Immunol Immunopathol* 1983;28:431-40.
10. Baur S, Fisher JM, Strickland RG, Taylor KB. Autoantibody-containing cells in the gastric mucosa in pernicious anaemia. *Lancet* 1968;2:887-94.
11. Irvine WJ. Immunologic aspects of pernicious anemia. *N Engl J Med* 1965;273:432-8.

12. Wall AJ, Whittingham S, Mackay IR, Ungar B. Prednisolone and gastric atrophy. *Clin Exp Immunol* 1968;3:359-66.
13. Jorge AD, Sanchez D. The effect of azathioprine on gastric mucosal histology and acid secretion in chronic gastritis. *Gut* 1973;14:104-6.
14. Karlsson FA, Burman P, Loof L, Mardh S. Major parietal cell antigen in autoimmune gastritis with pernicious anemia is the acid-producing H⁺,K⁺-adenosine triphosphatase of the stomach. *J Clin Invest* 1988;81:475-9.
15. Goldkorn I, Gleeson PA, Toh BH. Gastric parietal cell antigens of 60-90, 92, and 100-120 kDa associated with autoimmune gastritis and pernicious anemia: role of N-glycans in the structure and antigenicity of the 60-90-kDa component. *J Biol Chem* 1989;264:18768-74.
16. Toh BH, Gleeson PA, Simpson RJ, et al. The 60- to 90-kDa parietal cell autoantigen associated with autoimmune gastritis is a β subunit of the gastric H⁺/K⁺-ATPase (proton pump). *Proc Natl Acad Sci U S A* 1990;87:6418-22.
17. Callaghan JM, Khan MA, Alderuccio F, et al. α And β subunits of the gastric H⁺/K⁺-ATPase are concordantly targeted by parietal cell autoantibodies associated with autoimmune gastritis. *Autoimmunity* 1993;16:289-95.
18. Gleeson PA, Toh BH. Molecular targets in pernicious anaemia. *Immunol Today* 1991;12:233-8.
19. Pedersen PL, Carafoli E. Ion motive ATPases. I. Ubiquity, properties, and significance to cell function. *Trends Biochem Sci* 1987;12:146-50.
20. Pettitt JM, van Driel IR, Toh B-H, Gleeson PA. From coiled tubules to a secretory canaliculus: a new model for membrane transformation and acid secretion by gastric parietal cells. *Trends Cell Biol* 1996;6:49-53.
21. Hoedemacker PJ, Ito S. Ultrastructural localization of gastric parietal cell antigen with peroxidase-coupled antibody. *Lab Invest* 1970;22:184-8.
22. Callaghan JM, Toh B-H, Pettitt JM, Humphris DC, Gleeson PA. Poly-N-acetylglucosamine-specific tomato lectin interacts with gastric parietal cells: identification of a tomato-lectin binding 60-90 $\times 10^3$ M_r membrane glycoprotein of tubulovesicles. *J Cell Sci* 1990;95:563-76.
23. Irvine WJ, Davies SH, Delamore IW, Williams AW. Immunological relationship between pernicious anaemia and thyroid disease. *BMJ* 1962;2:454-6.
24. De Aizpurua HJ, Cosgrove LJ, Ungar B, Toh B-H. Autoantibodies cytotoxic to gastric parietal cells in serum of patients with pernicious anemia. *N Engl J Med* 1983;309:625-9.
25. Tanaka N, Glass VB. Effect of prolonged administration of parietal cell antibodies from patients with atrophic gastritis and pernicious anemia on the parietal cell mass and hydrochloric acid output in rats. *Gastroenterology* 1970;58:482-93.
26. de Aizpurua HJ, Ungar B, Toh B-H. Autoantibody to the gastrin receptor in pernicious anemia. *N Engl J Med* 1985;313:479-83.
27. Burman P, Mardh S, Norberg L, Karlsson FA. Parietal cell antibodies in pernicious anemia inhibit H⁺,K⁺-adenosine triphosphatase, the proton pump of the stomach. *Gastroenterology* 1989;96:1434-8.
28. de Aizpurua HJ, Toh BH, Ungar B. Parietal cell surface reactive autoantibody in pernicious anemia demonstrated by indirect immunofluorescence. *Clin Exp Immunol* 1983;52:341-9.
29. Masala C, Smurra G, Di Prima MA, Amendolea MA, Celestino D, Salzano F. Gastric parietal cell antibodies: demonstration by immunofluorescence of their reactivity with surface of the gastric parietal cells. *Clin Exp Immunol* 1980;41:271-80.
30. Kojima A, Prehn RT. Genetic susceptibility to post-thymectomy autoimmune disease in mice. *Immunogenetics* 1981;14:15-27.
31. Smith H, Lou Y-H, Lacy P, Tung KSK. Tolerance mechanism in experimental ovarian and gastric autoimmune diseases. *J Immunol* 1992;149:2212-8.
32. Gleeson PA, Toh BH, van Driel IR. Organ-specific autoimmunity induced by lymphopenia. *Immunol Rev* 1996;149:97-125.
33. Sakaguchi S, Sakaguchi N. Organ-specific autoimmune disease induced in mice by elimination of T cell subsets. V. Neonatal administration of cyclosporin A causes autoimmune disease. *J Immunol* 1989;142:471-80.
34. Ahmed SA, Penhale WJ. Pathological changes in inbred strains of mice following early thymectomy and irradiation. *Experientia* 1981;37:1341-3.
35. Barrett SP, Toh BH, Alderuccio F, van Driel IR, Gleeson PA. Organ-specific autoimmunity induced by adult thymectomy and cyclophosphamide-induced lymphopenia. *Eur J Immunol* 1995;25:238-44.
36. Martinelli TM, van Driel IR, Alderuccio F, Toh BH. Analysis of mononuclear cell infiltrate and cytokine production in murine autoimmune gastritis. *Gastroenterology* 1996;110:1791-802.
37. Suri-Payer E, Kehn PJ, Cheever AW, Shevach EM. Pathogenesis of post-thymectomy autoimmune gastritis: identification of anti-H/K adenosine triphosphatase-reactive T cells. *J Immunol* 1996;157:1799-805.
38. Jones CM, Callaghan JM, Gleeson PA, Mori Y, Masuda T, Toh BH. The parietal cell autoantigens recognized in neonatal thymectomy-induced murine gastritis are the α and β subunits of the gastric proton pump. *Gastroenterology* 1991;101:287-94. [Erratum, *Gastroenterology* 1992;102:1829.]
39. Alderuccio F, Toh BH, Tan SS, Gleeson PA, van Driel IR. An autoimmune disease with multiple molecular targets abrogated by the transgenic expression of a single autoantigen in the thymus. *J Exp Med* 1993;178:419-26.
40. Alderuccio F, Gleeson PA, Berzins SP, Martin M, van Driel IR, Toh BH. Expression of the gastric H⁺/K⁺-ATPase α -subunit in the thymus may explain the dominant role of the β -subunit in the pathogenesis of autoimmune gastritis. *Autoimmunity* 1997;25:167-75.
41. Nishio A, Hosono M, Watanabe Y, Sakai M, Okuma M, Masuda T. A conserved epitope on H⁺,K⁺-adenosine triphosphatase of parietal cells discerned by a murine gastritogenic T-cell clone. *Gastroenterology* 1994;107:1408-14.
42. Barrett SP, Gleeson PA, de Silva H, Toh BH, van Driel IR. Interferon- γ is required during the initiation of an organ-specific autoimmune disease. *Eur J Immunol* 1996;26:1652-5.
43. Barrett SP, van Driel IR, Tan SS, Alderuccio F, Toh BH, Gleeson PA. Expression of a gastric autoantigen in pancreatic islets results in non-destructive insulinitis after neonatal thymectomy. *Eur J Immunol* 1995;25:2686-94.
44. Asano M, Toda M, Sakaguchi N, Sakaguchi S. Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. *J Exp Med* 1996;184:387-96.
45. Taguchi O, Takahashi T. Administration of anti-interleukin-2 receptor α antibody in vivo induces localized autoimmune disease. *Eur J Immunol* 1996;26:1608-12.
46. Bonomo A, Kehn PJ, Shevach EM. Post-thymectomy autoimmunity: abnormal T-cell homeostasis. *Immunol Today* 1995;16:61-7.
47. Scarff KJ, Pettitt JM, van Driel IR, Gleeson PA, Toh BH. Immunisation with gastric H⁺/K⁺-ATPase induces a reversible autoimmune gastritis. *Immunology* 1997;92:91-8.
48. Seetharam B, Alpers DH, Allen RH. Isolation and characterization of the ileal receptor for intrinsic factor-cobalamin. *J Biol Chem* 1981;256:3785-90.
49. Delva PL, MacDonell JE, MacIntosh OC. Megaloblastic anemia occurring simultaneously in white female monozygotic twins. *Can Med Assoc J* 1965;92:1129-31.
50. Whittingham S, Mackay IR, Tait BD. Immunogenetics of pernicious anemia. In: Farid NR, ed. *Immunogenetics of autoimmune diseases*. London: CRC Press, 1991:215-38.
51. Whittingham S, Mackay IR. Pernicious anemia and gastric atrophy. In: Rose NR, Mackay IR, eds. *The autoimmune diseases*. Orlando, Fla.: Academic Press, 1985:243-66.
52. Herbert V. Vitamin B-12. In: Ziegler EE, Filer LJ Jr, eds. *Present knowledge in nutrition*. 7th ed. Washington, D.C.: ILSI Press, 1996:191-205.
53. Hsing AW, Hansson L-E, McLaughlin JK, et al. Pernicious anemia and subsequent cancer: a population-based cohort study. *Cancer* 1993;71:745-50.
54. Sjoblom SM, Sipponen P, Jarvinen H. Gastroscopic follow up of pernicious anaemia patients. *Gut* 1993;34:28-32.
55. Cowling DC, Strickland RG, Ungar B, Whittingham S, Rose WM. Pernicious-anaemia-like syndrome with immunoglobulin deficiency. *Med J Aust* 1974;1:15-7.
56. McIntyre OR, Sullivan LW, Jeffries GH, Silver RH. Pernicious anemia in childhood. *N Engl J Med* 1965;272:981-6.
57. Katz M, Lee SK, Cooper BA. Vitamin B₁₂ malabsorption due to a biologically inert intrinsic factor. *N Engl J Med* 1972;287:425-9.
58. Muller HK, McGiven AR, Nairn RC. Immunofluorescent staining of rat gastric parietal cells by human antibody unrelated to pernicious anaemia. *J Clin Pathol* 1971;24:13-4.
59. Strickland RG, Hooper B. The parietal cell heteroantibody in human sera: prevalence in a normal population and relationship to parietal cell autoantibody. *Pathology* 1972;4:259-63.
60. Bardhan KD, Hall JR, Spray GH, Callender STE. Blocking and binding autoantibody to intrinsic factor. *Lancet* 1968;1:62-4.
61. Waters HM, Dawson DW, Howarth JE, Geary CG. High incidence of type II autoantibodies in pernicious anaemia. *J Clin Pathol* 1993;46:45-7.