Invited Review

Immunopathology of autoimmune gastritis: Lessons from mouse models

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Summary. Autoimmune gastritis in humans is a chronic inflammatory disease of the stomach accompanied by specific destruction of gastric parietal and zymogenic cells resulting in pernicious anemia. Human gastritis can be accurately reproduced in mice and is characterised by autoantibodies to the *a-* and ß-subunits of the gastric H/K ATPase (the enzyme responsible for gastric acid secretion) and cellular destruction of parietal and zymogenic cells within the gastric gland. Studies with these mouse models have given us our current concepts of the immunopathogenesis of the gastritis. Mouse models have shown that a T cell response is generated to the a- and ß-subunits of the H/K ATPase and that an immune response to the B-subunit seems to be required for disease initiation. Using these models, we have defined key events associated with a damaging autoimmune response to the gastric H/K ATPase. The mechanisms associated with the cellular destruction associated with autoimmune gastritis are not know, but may involve signaling through death inducing pathways such as the Fas/FasL and TNF/TNFR pathways. This knowledge should permit us to develop strategies to prevent and treat the gastritis.

Key words: Autoimmune gastritis, Autoimmunity, Transgenic mice

Autoimmune gastritis and percinicious anemia

Autoimmune gastritis in humans is an organ-specific autoimmune disease belonging to a group of organ-specific diseases known as the "autoimmune endocrinopathies". These include diseases mainly involving endocrine organs such as the stomach (autoimmune gastritis and pernicious anaemia), thyroid (thyroiditis), the islets of Langerhans (autoimmune diabetes) and the adrenal cortex (Addison's disease). Human autoimmune gastritis (chronic atrophic gastritis

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type A) is the underlying cause of pernicious anaemia. The gastritis is restricted to fundus and the body of the stomach which contain the acid-secreting gastric parietal cells. This is in contrast with non-autoimmune (chronic atrophic gastritis type B) gastritis which involves the antrum of the stomach as well as the fundus and body, and which is typically associated with *Helicobacter pylori* infections. Human autoimmune gastritis is associated with circulating autoantibodies to gastric parietal cells and to intrinsic factor. However, the distinction between the two types of gastritis based on the presence or absence of circulating autoantibodies may not be absolute with recent findings of parietal cell autoantibodies associated with *Helicobacter pylori* infection (Appelmelk et al., 1998).

It has recently been reported that up to 2% of persons of over the age of 60 may have undiagnosed pernicious anaemia (Carmel, 1996). The disease is not restricted to any particular racial group because it has been reported in Northern Europeans, Black and Latin Americans (Carmel, 1992). The natural history of autoimmune gastritis leading to pernicious anaemia suggests that the progression of disease is over a long period of time; probably in the order of 20-30 years. Autoimmune gastritis can be predicted by the presence of circulating autoantibodies to parietal cells whereas antibodies to intrinsic factor is typically associated with pernicious anemia (Toh et al., 1997). Histologically, stomachs from patients with pernicious anaemia show a mononuclear cell infiltrate in the submucosa which extends into the lamina propria between the gastric glands. The gastric gland is composed of numerous cell types including the parietal, zymogenic (chief) and surface mucous cells (Fig. 1). In addition, endocrine and mucous neck cells are also present. Parietal cells are responsible for acidification of the gastric juices by the pumping of H+ ions into the stomach lumen. The exchange of H+ ions for K^+ ions is performed by the gastric H/K ATPase which is located on the secretory canaliculus of the parietal cell which forms a continuous membrane with the apical surface of the parietal cell (Pettitt et al., 1995). The zymogenic cells secrete pepsingogen which is converted to the digestive enzyme pepsin in the acidic environment of the stomach. The

Table 1. Experimental conditions which result in autoimmune gastritis in mice.

EXPERIMENTAL PROCEDURE	REFERENCES
Lymphopenic	
1. Neonatal thymectomy	Kojima and Prehn, 1981; Tung et al., 1987a; Gleeson and Toh, 1991
2. Neonatal administration of cyclosporin A	Sakaguchi and Sakaguchi, 1989
3. High dose fractionated total lymphoid irradiation	Sakaguchi et al., 1994a
4. Adult thymectomy combined with cyclophoshamide treatment	Barrett et al., 1995
5. Single TCR α-chain transgenic mice	Sakaguchi et al., 1994b
6. CD4+ CD25- T cell transfer to T cell deficient mice	Sakaguchi et al., 1985, 1995; Smith et al., 1992
7. Thymus/thymocyte transfer to T cell deficient mice	Sakaguchi and Sakaguchi, 1988; Smith et al., 1992; Alderuccio et al., 1993
Non-lymphopenic	
8. Immunisation with autoantigen	Wantanabe et al., 1977; Kontani et al., 1992; Alderuccio et al., 1997; Scarff et al., 1997; Claeys et al., 1997
9. Spontaneous development in C3H/He mice	Alderuccio and Toh, 1998

surface mucous cells are located at the interface of the stomach wall and lumen and secrete protective bicarbonate and mucus to protect the gastric lining. Endocrine cells produce a variety of hormones which act upon cells within the gastric mucosa (Simonsson et al., 1988). Mucous neck cells comprise a population of cells which include stem cells responsible for the generation of the cells types found in the gastric glands.

Parietal cell antibodies react with the gastric H/K ATPase

A major advance in our understanding of the immunopathology of autoimmune gastritis was the demonstration that the gastric H/K ATPase (or proton pump) is the autoantigen reactive with human parietal cell autoantibody (Karlsson et al., 1988; Goldkorn et al., 1989; Toh et al., 1990; Callaghan et al., 1993). The gastric H/K ATPase is a heterodimer compring a 100 kDa α-subunit and a 60-90 kDa ß-subunit located in the secretory canaliculi of the cell (Pettitt et al., 1995) (Fig. 2). The α-subunit is the catalytic subunit which is phosphorylated during each reaction cycle in which extracellular K^+ ions are exchanged for intracellular H^+ ions. This process can occur over a million fold gradient

and consumes large amounts of energy, which explains the large number of mitochondria present in parietal cells (Helander and Keeling, 1993). The ß-subunit of the H/K ATPase is highly glycosylated and aids in the stabilization of the α-subunit (Geering, 1991; Jaunin et al., 1993) and is required for acid secretion (Scarff et al, 1999). In human autoimmune gastritis and pernicious anaemia, as well as in animals models of autoimmune gastritis (see below), autoantibodies are generated to both the α- and ß-subunit of the gastric H/K ATPase (Callaghan et al., 1993).

### Animal models of human autoimmune gastritis

Our understanding of the immunopathology of autoimmune gastritis has come from the study of mouse models. Several mouse models of experimental autoimmune gastritis (EAG) have been described. These mouse models can be divided into two broad groups on the basis of whether they involve a state of lymphopenia or not (Table 1). The best characterised lymphopenic model is that induced by surgical removal of the thymus from 2-4 day old BALB/c mice (Fig. 3A). This procedure of neonatal thymectomy induces autoimmune
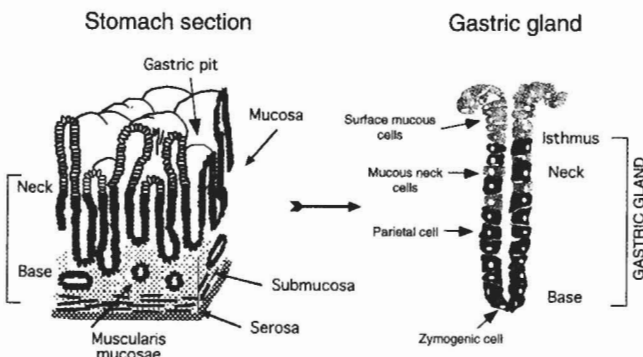
Fig. 1. Schematic representation of stomach section and gastric glands which comprise the gastric mucosa. The composition of the gastric gland includes parietal cells, zymogenic cells, mucous neck cells and surface mucous cells as illustrated.
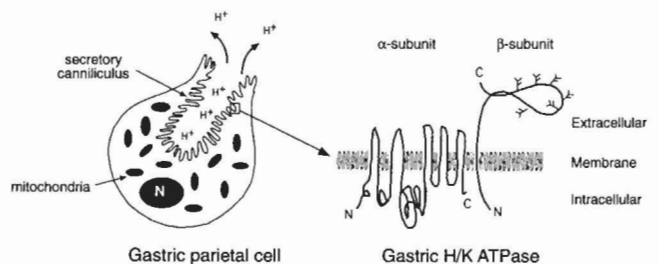

Fig. 2. The gastric parietal cell secretes H+ ions into the stomach lumen in exchage for K+ ions. The cytoplasm of these cells contains an extensive network of secretory membranes in which the H/K ATPase heterodimer is located. Transport of H+ ions across parietal cell membrane requires energy, explaining why parietal cells are mitochondria rich. The gastric H/K ATPase is composed of a 100 kDa catalytic α-subunit and a 60-90 kDa highly glycosylated ß-subunit. Ion transport is conducted through the α-subunit while the ß-subunit confers stability to the assembly of the α- and ß-subunits (Scarff et al., 1999).
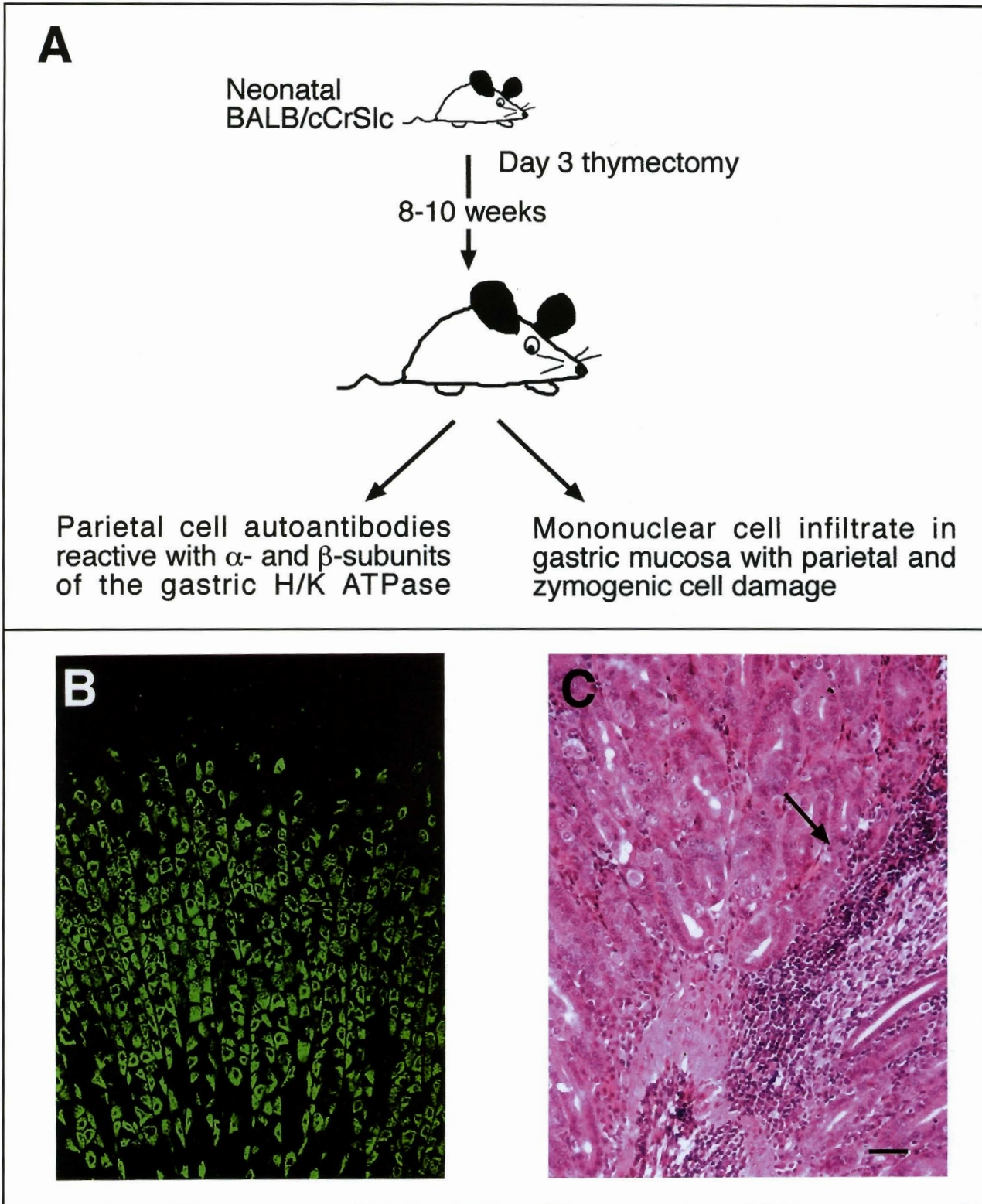
Fig. 3. A. Experimental autoimmune gastritis can be induced in BALB/c and BALB/cCrSlc mice by removing the thymus 3 days after birth. Eight to ten weeks following thymectomy the presence of gastritis can be predicted by circulating autoantibodies to gastric parietal cells and confirmed by the presence of a mononuclear cell infiltrate in the gastric mucosa. **B.** Reactivity of serum from a mouse with EAG on normal mouse stomach section. Parietal cells are stained green displaying reactivity with intracellular parietal cell membranes. **C.** Haemotoxylin and eosin staining of formalin fixed, paraffin-embedded gastritic stomach. Gastritis is characterised by a prominent mononuclear cell infiltrate in the lamina propria between gastric glands (arrowed) accompanied by loss of parietal and zymogenic cells and mucosal hypertrophy. Bar: 100 μm.

gastritis in 40-60% of BALB/c mice and to a lesser extent autoimmune oophoritis or autoimmune orchitis (Kojima and Prehn, 1981; Fukuma et al., 1988). More recently, we have favoured the use of a BALB/cCrSlc substrain obtained from Tohru Masuda in which greater than 90% of mice develop autoimmune gastritis following neonatal thymectomy (Alderuccio et al., 1995). A peculiarity of neonatal thymectomy is that the induced autoimmune disease is strain specific with different strains of mice developing different autoimmune diseases (Kojima and Prehn, 1981). These observations suggest a genetic basis for susceptibility of the development of an autoimmune disease affecting a particular organ following neonatal thymectomy. This suggestion is supported by the mapping of gastritis-susceptibility genes to two regions on the distal arm of chromosome 4, designated Gasa1 and Gasa2 (Silveira et al., 1999). Neonatal thymectomy of BALB/C and BALB/cCrSlc mice induces parietal cell specific autoantibodies (Fig. 3B) and a mononuclear cell infiltrate within the gastric mucosa (Fig. 3C, 4B). The gastritis is associated with parietal and zymogenic cell loss and hypertrophy of the gastric mucosa (Tung et al., 1987b; Fukuma et al., 1988). Furthermore, the parietal cell autoantibodies are directed to the α- and ß-subunit of the gastric H/K ATPase (Jones et al., 1991). Apart
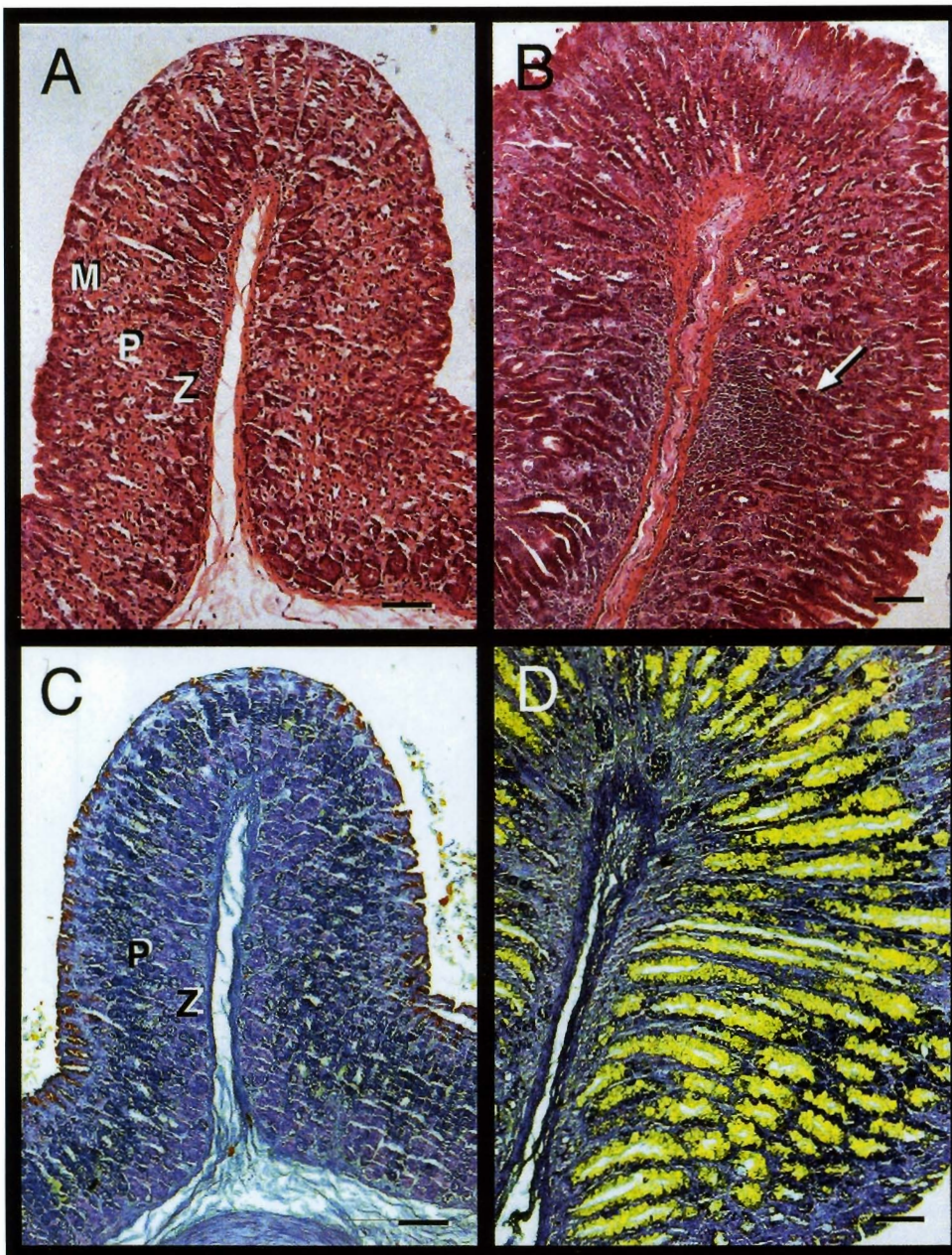
Figure 4. Histological staining of paraffin-embedded normal **(A, C)** and gastritic **(B, D)** stomachs by Haemotoxylin and Eosin (A, B) or by a modified Maxwell stain (C, D). Normal stomach (A) shows zymogenic (Z), parietal (P) and surface mucous cells (M). Gastritic stomach (B) are characterised by mononuclear cell infiltrates (arrowed). Normal stomach (C) identifies parietal cells which stain blue (P) from zymogenic cells which stain purple-pink (Z). In gastritic stomachs (D), parietal and zymogenic cell staining is reduced due to loss of these cells and replacement by mucus secreting cells which stain yellow. Bar 100 μm.

Table 2. Features of human autoimmune gastritis and pernicious anaemia compared to mouse autoimmune gastritis .

HUMAN GASTRITIS	MOUSE GASTRITIS	REFERENCE
1. Autoantibodies reactive with parietal cell H/K ATPase	Yes	Tung et al., 1987a; Fukuma et al., 1988; ; Mori et al., 1989; Jones et al., 1991
2. Autoantibodies reactive with intrinsic factor	Not Definitive	Sakaguchi et al., 1985; Tung et al., 1987a; Fukuma et al., 1988
3. Parietal and zymogenic cell loss from gastric mucosa	Yes	Kojima et al., 1980; Scarff et al., 1997; Judd et al., 1999
4. Mononuclear cell infiltrate within gastric mucosa	Yes	Tung et al., 1987b; Fukuma et al., 1988; Alderuccio et al., 1995
5. Achlorhydria (lack of gastric acid)	Yes	Sakaguchi et al., 1994a
6. Association with other autoimmune diseases	Yes	Kojima and Prehn, 1981; Sakaguchi et al., 1985; Sakaguchi and Sakaguchi, 1990; Gleeson et al., 1996;
7. Increased serum gastric levels	Yes	Kubota et al., 1986
8. Gastric cancer predisposition	Unknown	
9. Megablastic anaemia	Yes	Kojima et al., 1980
10. Atrophy of gastric mucosa	No*	

*: in contrast to human autoimmune gastritis, mouse models of autoimmune gastritis develop a distinct hypertrophy of the gastric mucosa which is probably the result of accumulation of immature cells within the gastric glands (Judd et al., 1999).

from hypertrophy of the gastric mucosa which is not associated with human autoimmune gastritis, many of the major features of human and murine autoimmune gastritis are identical (Table 2). As such EAG has proven to be an excellent model for the study of autoimmune gastritis and organ-specific autoimmunity in general (Gleeson et al., 1996; Toh et al., 1997).

The pathology of EAG is similar to that of human autoimmune gastritis. The chronic inflammatory lesion within the gastric mucosa (Fig. 4) is associated with a mononuclear cell infiltrate and destruction of parietal and zymogenic cells. As a result, there is expansion of smaller immature cells within the gastric gland which are rich in mucus substances (Scarff et al., 1997; Judd et al., 1999). Histologically, regions of tissue destruction and cell replacement can be readily identified by the use of a modified Maxwell's stain (Beinborn et al., 1993; Scarff et al., 1997) (Fig. 4C,D). The procedure uses a series of dyes which allow the differential identification of parietal, zymogenic and mucus secreting cells. As illustrated in Figure 4C and D, these three regions are easily distinguished. Parietal cells stain blue due to the affinity of luxol fast blue for the phospholipids in the secretory membranes and mitochondria. Zymogenic cells are rich in rough endoplasmic reticulum and stain purple-pink due to affinity of pyronin for RNA. Finally, alcian yellow identifies areas rich in mucus by staining yellow the carbohydrates present in surface mucous cells and the immature mucus rich cells which in the gastric glands of mice with EAG.

Experimental autoimmune gastritis is a CD4+ T cell mediated disease. This has been well documented in both transfer and depletion studies (Sakaguchi et al., 1985; Smith et al., 1992). CD8+ T cells play no role in the induction of autoimmune gastritis (De Silva et al., 1998). This is supported by studies that have detailed the genesis of EAG by examining the cells that infiltrate the gastric lesion of mice. The first signs of an inflammatory infiltrate are detected at about 4 weeks post-thymectomy in which there is an influx of CD4+ T cells and

macrophages (Martinelli et al., 1996) which increases with time. Within the gastric lesions, a number of T cell secreted cytokines can be identified by immunohistochemistry including IFN-γ, IL-10, TNFα and GM-CSF but not IL-4 (Martinelli et al., 1996). These findings together with the observations that gastric T cells produced more IFN-γ than normal mice and that neutralising antibodies to IFN-γ prevents EAG (Barrett et al., 1996), suggest that EAG is a Th1 mediated disease. There is little change in the number of gastric-infiltrating CD8+ T cells over the course of the disease. B cells are also observed in the gastritis lesion and become greatly accentuated in the latter stages of disease (Martinelli et al., 1996). This coincides with the observation of follicles within the gastric mucosa which are surrounded by T cells and may represent the generation of functional lymphoid follicles (Ludewig et al., 1998).

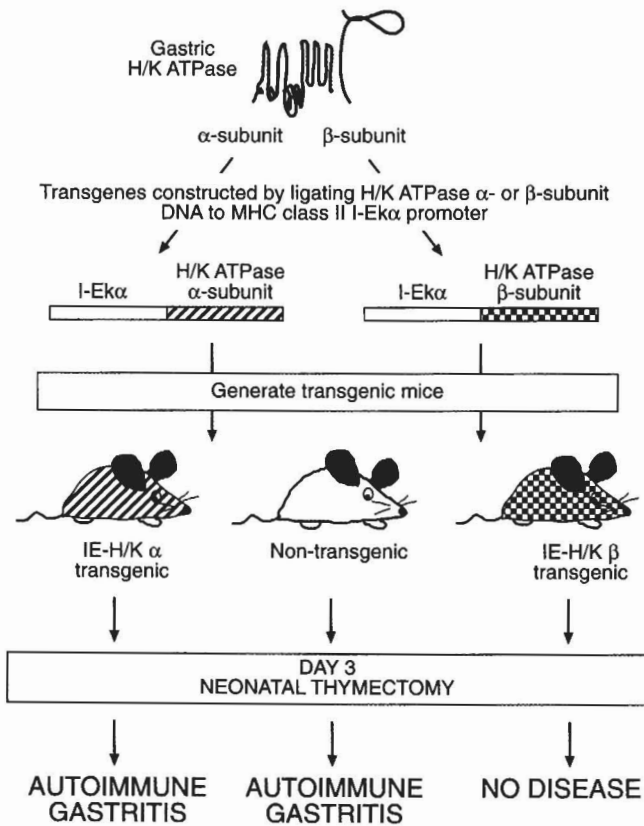### The gastric H/K ATPase is the causative antigen of gastritis

Our knowledge of the causative T cell autoantigen of EAG came through the use of transgenic technology. We generated transgenic mice in which the H/K ATPase α- or ß-subunit were ectopically expressed in the thymus under the control of the MHC class II I-E^kα promoter (Fig. 5). This strategy was applied so as to render autoreactive T cells developing within the thymus tolerant when they encounter these subunits presented by thymic MHC class II bearing antigen presenting cells (Blackman et al., 1990). Mice were then tested for development of EAG following neonatal thymectomy. Transgenic expression of the H/K ATPase ß-subunit in the thymus (IE-H/Kß tg) completely abrogated the induction of EAG following neonatal thymectomy (Alderuccio et al., 1993). In contrast, transgenic expression of the H/K ATPase α-subunit did not alter the course of disease (Alderuccio et al., 1997). It should be noted that whereas the H/K ATPase α subunit is

expressed in the normal thymus the ß subunit is not. These IE-H/Kß transgenic mice are also resistent to the initiation of gastritis by other methods including immunisation with autoantigen (Alderuccio et al., 1997), adult thymectomy combined with cyclophosphamide treatment (Barrett et al., 1995) and in single TCRα-chain transgenic mice which develop gastritis on a BALB/c background (F. Alderuccio, unpublished data).

These results suggest that an immune response to the H/K ATPase ß-subunit is crucial for disease induction. This has recently been confirmed in a knock out mouse model in which expression of the gastric H/K ATPase ß-subunit has been prevented by gene targeting. H/K ATPase ß-subunit knock out mice do not develop

Fig. 5. Transgenic models demonstrating that an immune response to the H/K ATPase ß-subunit is required for initiation of gastritis. Transgenic mice were produced expressing either the gastric H/K ATPase α- or ß-subunit under control of the MHC class II I-Ekα promoter, to drive expression of these subunits in the thymus (note that thymuses from non transgenic mice express the gastric H/K ATPase α subunit but not the ß subunit). Transgenic mice were subjected to neonatal thymectomy and assessed for development of gastritis at 10 weeks. IE-H/Kß-transgenic were resistant to gastritis induction whereas, IE-H/Kα-transgenic mice and non-transgenic littermates develop gastritis at the expected frequency. A second autoimmune disease, oophoritis which is also induced in BALB/c mice by neonatal thymectomy was not abrogated in IE-H/Kß-transgenic mice indicating the specificity of the response. These studies indicate that an immune response to the H/K ATPase ß-subunit is required for initiation of autoimmune gastritis.

EAG following neonatal thymectomy (K Scarff et al., unpublished data) confirming the importance of the ß-subunit in disease initiation. A major gastritogenic epitope on the H/K ATPase ß-subunit has been indentified and maps to a 14 amino acid region in the C-terminus (de Silva et al., 1999). However, it is clear from the literature, that T cell responses are generated to both the α- and ß-subunits (Nishio et al., 1994; Katakai et al., 1997; Suri-Payer et al., 1999) with perhaps the response to the α-subunit dominating the autoimmune response in the established disease (Suri-Payer et al., 1999). Together, these findings suggest that the pathogenesis of EAG is initiated by an immune response to the gastric H/K ATPase ß-subunit with subsequent spreading of the immune response to include the H/K ATPase α-subunit. Epitope spreading has been observed in other models of autoimmunity (Lehmann et al., 1992; Tisch et al., 1993) and more recently there is evidence that the immune response to the initiating antigen in EAE is diminished or lost with time (Tuohy et al., 1999).

We have recently generated TCR transgenic mice with specificity for the major gastritogenic peptide H/Kß261-274 (Alderuccio et al., 2000). The T cell repertoire in these TCR transgenic should be dominated by T cells reactive with the H/K ATPase ß-subunit peptide. While we might have expected these mice to develop florid gastritis, this was not the case. In fact only a minority of mice developed spontaneous EAG, while the lymphocytes from all the TCR transgenic mice tested proliferated in vitro to the H/Kß peptide. Findings such as these highlight the role of tolerance mechanisms which prevent the immune system from widespread autoaggression. These mice should prove very useful in further defining the mechanisms which induce autoimmunity and immunological tolerance to the gastritogenic peptide.

Parietal cell destruction in autoimmune gastritis

Relatively little is known about the mechanisms associated with the cell destruction observed in EAG. While the pathogenic lesion associated with EAG is mediated by CD4+ T cells and increased apoptosis, how the damage is initiated and whether other non-lymphocytic cell types are involved is not known. However, detailed analysis of gastritic stomachs has shown that the depletion of parietal and zymogenic cells associated with gastritis is accompanied by an accumulation of immature, rapidly dividing cells of which the majority die by apoptosis (Judd et al., 1999). The hypertrophy associated with EAG is due to the accumulation of these immature cells in which the normal development of parietal and zymogenic cells appear to be blocked. It remains possible that the associated depletion of zymogenic cells from gastritic stomachs may not be entirely immunological in nature but rather a consequence of the disease since they may stem from a common precursor within the gastric mucosa (Judd et al., 1999).

Recently, the role of Fas (CD95) and TNFα as mediators of cell death has been examined in several models of autoimmunity and in human disease. Fas/FasL has been implicated in several autoimmune disorders (reviewed by De Maria and Testi (De Maria and Testi, 1998)) including diabetes in NOD mice (Chervonsky et al., 1997; Itoh et al., 1997) and EAE (Sabelko et al., 1997; Waldner et al., 1997). Fas has also been implicated in EAG in which Fas expression has been observed on parietal cell from gastritic stomachs (Nishio et al., 1996). We have also observed the upregulation of Fas in parietal cells of gastritic mice and the absence of EAG in neonatally thymectomised lpr BALB/cCrSlc mice (Marshall et al., unpublished data). Therefore, the expression of Fas on parietal cells may induce cell death and be triggered by FasL on infiltrating T cells, FasL released by proteolysis from T cell membranes or FasL expressed on adjacent epithelial cells within the gastric gland (Fig. 6). While this data supports a role for Fas in autoimmune disease a recent study has questioned this role (Allison and Strasser, 1998).

The TNF family comprises TNFα, TNFβ and lymphotoxin ß and signals through two receptors, TNFRI (p55) and TNFRII (p75). While TNFα signaling can result in cell death, TNFα also has pro-inflammatory activity resulting in upregulation of adhesion molecules and induction of other pro-inflammatory cytokines. TNFα has been identified in the gastric lesion of mice with EAG (Martinelli et al., 1996), but little is known of its role in the pathogenesis of autoimmune gastritis. In human patients, the use of neutralising antibody to TNFα in treating rheumatoid arthritis (Feldmann et al., 1997) indicates that TNFα is involved in tissue destruction. This is further supported in animal models in which mice transgenically expressing TNFα in the CNS develop a more severe, non-remitting form of EAE while mice lacking TNFα have a delayed onset of EAE (Kroner et al., 1997; Taupin et al., 1997). At present, the role of TNFα in EAG is not known but may have a role in tissue destruction (Fig. 6). However, it has been observed that TNFα can have opposing effects depending on the timing of administration. For example, in the NOD mouse model of diabetes, TNFα administered to neonatal mice accelerates disease, but if administered to adult mice, disease is suppressed (Cope et al., 1997). Clearly more studies are needed to understand this paradoxical effect.

Regulatory CD4 T cells

While central tolerance of autoreactive T cells effected through clonal deletion within the thymus is well established (Kruisbeek and Amsen, 1996), the mechanisms for the maintainance of tolerance to self antigens in the periphery remains largely unknown. Proposed mechanisms of peripheral tolerance include clonal deletion, anergy, ignorance and regulation (Kruisbeek and Amsen, 1996; Mason and Powrie, 1998). Recently, the study of immunoregulation by CD4+CD25+ T cells has become a topic of much interest. Sakaguchi originally showed that pathogenic CD4 T cells reside in the CD25- population since depletion of the CD25+ subset from pooled spleen and lymph node preparations from normal mice rendered the CD25- cells pathogenic when transferred to T cell deficient hosts (Sakaguchi et al., 1995). On the other hand, the regulatory cells appear to reside in the CD4+CD25+ population because EAG can be prevented by this populaton following neonatal thymectomy or by co-transfer of this population together with the pathogenic CD4+CD25- cells into T cell-deficient mice (Suri-Payer et al., 1998). Thus, the normal T cell repertoire appears to be composed of both pathogenic and regulatory CD4+ T cells. CD4+CD25+ regulatory T cells are positively selected within the thymus where they comprise 5-10% of the mature CD4+CD8- T cells (Itoh et al., 1999). They appear to be a unique lineage of cells selected within the thymus and cannot be generated by inducing CD25 expression on naïve T cells (Suri-Payer et al., 1998). The finding that CD4+CD25+ T cells do not leave the thymus until 3 days of age supports the hypothesis that the spectrum of autoimmune diseases observed in neonatal thymectomy mouse models is due to the absence of these cells (Asano et al., 1996); although this has been disputed (Suri-Payer et al., 1999).
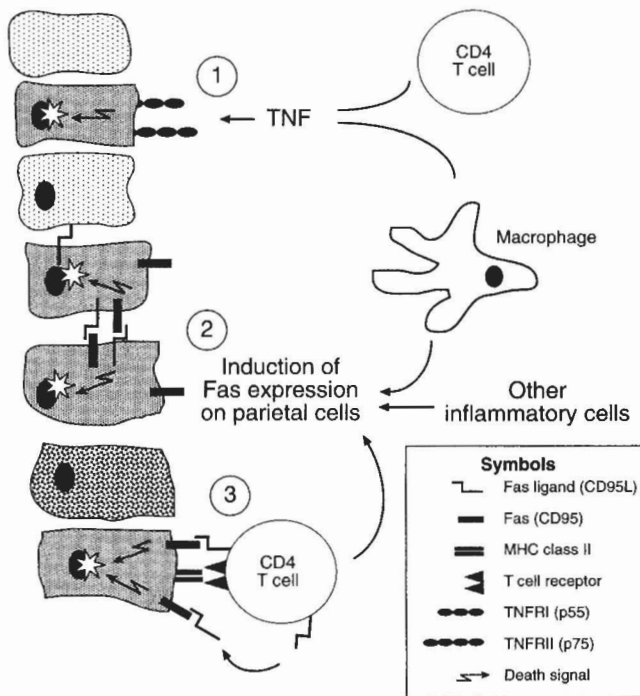
Fig. 6. Speculative models of parietal cell death in gastritis initiated by TNF and/or FasL. Parietal cell death may be initiated by one or more of the following mechanisms: (1) TNF released by CD4+ T cells and macrophages engage TNFRI and RII on parietal cells; (2) FasL present on parietal cells or (3) on CD4 T cells, or soluble Fas L released from CD4 T cell membranes, engage Fas upregulated in gastritic parietal cells by as yet unknown mechanisms.

Recently, elegant in vitro studies have given us insights into the nature of the CD4+CD25+ regulatory cells (Takahashi et al., 1998; Thornton and Shevach, 1998; Itoh et al., 1999). The CD4+CD25+ regulatory cells are naturally anergic and do not proliferate following stimulation with IL-2, con A, anti-CD3 or anti-CD28 antibody (Takahashi et al., 1998; Thornton and Shevach, 1998; Itoh et al., 1999). In vitro, CD4+CD25+ cells suppress the proliferation of CD4+CD25- following stimulation with conA or soluble anti-CD3. For their suppressive effect, CD4+CD25+ cell require signaling through the TCR and cell-to-cell contact with a third party antigen presenting cell (APC) (Thornton and Shevach, 1998; Itoh et al., 1999). While it is still not clear how the CD4+CD25+ cells exert their effect, studies have shown that suppression is not mediated by IL-4, IL-10, TGFß or other soluble factors (Thornton and Shevach, 1998; Itoh et al., 1999). In addition, if suppression is mediated through competition on the surface of the APC for antigen or co-stimulation, it does not involve CD28 or CD40L since CD4+CD25+ T cells from CD28-/- and CD40-/- mice still display suppressive activity (Thornton and Shevach, 1998).

The generation of CD4+ regulatory cells within the thymus seems to require the expression of endogenously rearranged TCRs. This is evident in the TCR transgenic model for EAE in which mice develop spontaneous autoimmune encephalomyelitis on a Rag1-/- or TCRα-chain -/- background where there are no endogenously

rearranged TCR α-chains (Olivares-Villogomez et al., 1998; Van de Keere and Tonegawa, 1998). Similarly, Sakaguchi has also shown in TCR-transgenic mice that thymic CD4+CD25+ cells contain a high proportion of cells expressing endogenous TCR α-chains (Itoh et al., 1999) and we have found that the CD4+CD25+ thymic population is not generated in TCR-transgenic mice crossed onto TCRα-chain -/- mice (Alderuccio, unpublished data). It is still unclear how expression of endogenous TCR α-chains results in selection of these CD4+CD25+ regulatory cells. It may be non-specific effects which alter the TCR signaling of the cell during thymic selection such that it is not deleted and able to escape into the periphery. Alternatively, selection of the CD4+CD25+ cells is dependant on the TCR generated by expression of endogenous TCR α-chains which would indicate that specific ligands may be involved. Whatever the reason, phenotypic analysis of CD4+CD25+ cells suggests that they may be partially activated and recently selected (Itoh et al., 1999). It is clear that understanding the selection and mechanisms associated with CD4+CD25+ regulatory cell will continue to be an area of much interest. By understanding the mechanisms associated with regulation induced by these cells and their role in maintaining immune regulation, we can begin to device strategies aimed at enhancing or re-establishing tolerance in autoimmune diseases or damping their capacity to enhance the immune response to antigens.

Conclusions

In summary, our understanding of the initiation and progression of autoimmune gastritis has come a long way over the last 10 years. We propose that the gastritis is initiated by an autoimmune response to the H/K ATPase ß-subunit with subsequent intermolecular spreading to the H/K ATPase α-subunit. This knowledge has therapeutic implications. Thus, while limiting the immune response to the H/K ATPase ß-subunit may be a good strategy for preventing autoimmune gastritis, this is likely to have little effect in the established disease since the pathogenic T cell response is likely to be also driven by autoepitopes of the H/K ATPase α-subunit. In established gastritis therefore we will need to devise strategies for limiting the autoimmune response driven by both the α as well as the ß subunit.

EAG appears to be a disease mediated solely by Th1 type CD4+ T cells. There is no evidence of a role for CD8+ T cells or antibodies to the H/K ATPase in disease initiation. As yet, we do not know the mechanisms by which parietal cells are lost from the gastric mucosa in EAG. Preliminary results implicate a role for Fas/FasL and TNF/TNF receptor systems. Future studies using neutralising antibodies and mice deficient in components of these systems should help clarify their relative involvement in EAG.

The role of CD4+CD25+ regulatory cells in immune regulation will be an exciting area of research in the
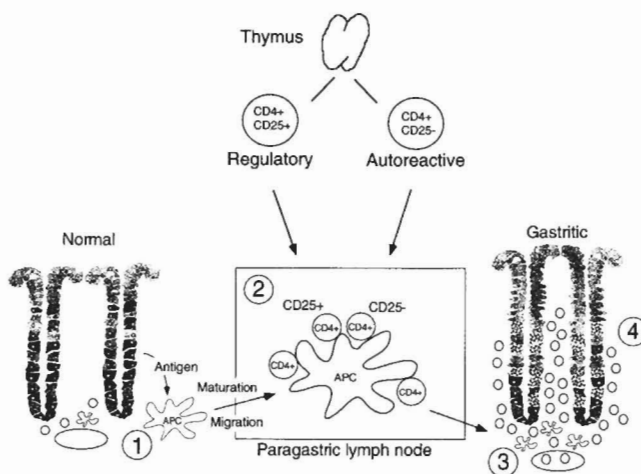
Fig. 7. Key stages associated with the development of autoimmune gastritis. (1) Following activation by a local inflammatory response, antigen presenting cells take up gastric H/K ATPase autoantigens, upregulate MHC-II and co-stimulatory molecules and migrate to local draining paragastric lymph node. (2) Activated antigen presenting cells in turn activate effector CD4 T cells and abrogate suppressor activity of regulatory CD4 T cells. (3) Activated effector CD4+ T cells leave the lymph node and home to the stomach where they bind to the endothelium of gastric blood vessels using adhesion molecules and traverse the gastric mucosa along a chemotactic gradiant provided by chemokines. (4) Once within the tissue, effector CD4+ T cells together with other inflammatory cells such as macrophages destroy parietal and zymogenic cells.

future. It is now known that these regulatory T cells require to contact the surface of a common antigen-presenting cell which presents self antigen to effector CD4+CD25- T cells. However precisely how this suppression is mediated is currently not known. Our understanding of how these CD4+CD25+ regulatory cells develop within the thymus is also not clear although we know from TCR transgenic mouse studies that these cells also express endogenous TCR α-chains. In the context of autoimmunity, future goals should include harnessing the regulatory powers of these cells to dampen the chronic inflammation associated with autoimmunity. The ability to clone these regulatory cells or inducing their production in vitro or in vivo should pave the way towards a better understanding of the way they function which in turn may lead to the development of strategies to inhibit or reverse the damaing autoimmune reaction. Similar strategies may also be adopted for the reversal of allergy or for the establishment of transplantation tolerance. Conversely, if these CD4+CD25+ regulatory T cells have a major role in suppressing the immune response to tumour neoantigens, then therapeutic strategies which result in their removal should boost the immune system to these antigens.

We have learnt from the EAG model that the autoimmune response to the gastric H/K ATPase is a dynamic process which progresses through a number of steps leading to the pathological lesions of autoimmune gastritis (Fig. 7). The critical requirement for the autoimmune response appears to be delivery of antigen to the regional paragastric lymph node. This most likely occurs via dendritic cells which pick up the antigen in the gastric mucosa and migrate to the local draining lymph node. Within the lymph node, autoreactive T cells are activated while the activity of regulatory T cells is abrogated. The activated T cells then migrate out of the lymph node and home to the stomach through the interaction of adhesion molecules on the activated lymphocytes and the endothelium of gastric blood vessels and signals delivered by chemokines. Research directed towards blocking one or more steps in the progression of the autoimmune reaction may also prove fruitful in limiting or reversing the damaging autoimmune response.

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