An Autoimmune Disease with Multiple Molecular Targets Abrogated by the Transgenic Expression of a Single Autoantigen in the Thymus

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Summary

Many autoimmune diseases are characterized by autoantibody reactivities to multiple cellular antigens. Autoantigens are commonly defined as targets of the autoimmune B cell response, but the role, if any, of these autoantigens in T cell–mediated autoimmune diseases is generally unknown. Murine experimental autoimmune gastritis is a $CD4^+$ T cell–mediated organ-specific autoimmune disease induced by neonatal thymectomy of BALB/c mice. The murine disease is similar to human autoimmune gastritis and pernicious anemia, and is characterized by parietal and chief cell loss, submucosal mononuclear cell infiltrates, and autoantibodies to the α and β subunits of the gastric H/K ATPase. However, the specificity of T cells that cause the disease is not known. To examine the role of the H/K ATPase in this T cell–mediated disease, transgenic mice were generated that express the β subunit of the H/K ATPase under the control of the major histocompatibility complex class II $I\text{-}E^k_\alpha$ promoter. We show that transgenic expression of the gastric H/K ATPase β subunit specifically prevents the onset of autoimmune gastritis after neonatal thymectomy. In addition, thymocyte transfer experiments suggest that tolerance of pathogenic autoreactive T cells is induced within the thymus of the transgenic mice. We conclude that the β subunit of the gastric H/K ATPase is a major T cell target in autoimmune gastritis and that thymic expression of a single autoantigen can abrogate an autoimmune response to multiple autoantigens.

Many autoimmune diseases are associated with autoantibodies to specific antigens. This is true for systemic autoimmune diseases such as systemic lupus erythematosus, Sjogren's Syndrome (1), and scleroderma (2, 3), and organ-specific autoimmune diseases such as diabetes (4), primary biliary cirrhosis (5), thyroiditis (6), and pernicious anemia (7). Another feature of the autoimmune response is that multiple self-molecules are often targeted within a particular disease such that an autoantibody profile for that disease can be described (2, 8). Apart from a few examples where autoantibodies to cell surface structures have been shown to be pathogenic, the role of autoantibodies in many autoimmune diseases is unclear (9).

There is strong evidence for the involvement of T cells in the pathogenesis of autoimmune diseases such as diabetes (10), experimental autoimmune uveitis (11), experimental autoimmune encephalomyelitis (12, 13), and experimental autoimmune gastritis (14–16). To define the mechanisms associated with T cell–mediated autoimmune diseases, it is of fundamental importance to first identify the antigens targeted by the T cells. Although many autoimmune diseases have multiple autoantibody targets, it is usually not known if these molecules are also T cell targets or whether the immune response to these antigens is involved in disease induction. Furthermore, immune responses in autoimmunity are usually most clearly defined at later stages of disease when pathology is well established. The predominant specificities of lymphocytes at this phase of disease may be quite different from the specificities that initiated autoimmunity during events that may have occurred months or even years before.

Autoimmune gastritis (chronic atrophic gastritis type A) is an organ-specific autoimmune disease of humans that is the underlying basis for pernicious anemia, the most common cause of vitamin B12 deficiency in White northern Europeans (17). The autoimmune lesion, confined to the fundus and body of the stomach, is typified by gastric mucosal atrophy, submucosal lymphocytic infiltrates, and loss of parietal and chief cells from the gastric mucosa (17). Circulating autoantibodies in autoimmune gastritis and pernicious anemia target molecules associated exclusively with the parietal cell (the α and β subunits of the gastric H/K ATPase complex and intrinsic factor) (16–19).

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Neonatal (day 2–4) thymectomy of BALB/c mice results in the induction of autoimmune gastritis and oophoritis with a frequency of 40–60 and 20–30%, respectively (20, 21). Murine experimental autoimmune gastritis shares many of the features displayed by human autoimmune gastritis, such as submucosal lymphocytic infiltrates, selective parietal and chief cell loss (21–23), and autoantibodies reactive with the α and β subunits of the gastric H/K ATPase (24–27). It has become a widely used model for the study of autoimmune gastritis and organ-specific autoimmune diseases (7, 16, 21, 26, 28). Murine experimental autoimmune gastritis is a T cell–mediated disease, since the transfer of $CD4^+$ T cells, but not serum, from diseased animals to nude or SCID mice is sufficient to initiate disease (14, 15).

We have shown that the B cell autoantigens in experimental autoimmune gastritis are also the α and β subunits of the gastric H/K ATPase complex (25). To define the T cell targets in this disease, we have produced transgenic mice that express the β subunit of the H/K ATPase under the control of the MHC class II I-E^k_α promoter (βH/K transgenic), resulting in widespread tissue expression of the β subunit, including the thymus. We show that transgenic expression of the β subunit alone prevents the production of autoantibodies to the α and β subunits of the H/K ATPase and the development of autoimmune gastritis manifested as gastric mononuclear cell infiltration after neonatal thymectomy. Thymocytes from βH/K-transgenic mice, in contrast to thymocytes from normal BALB/c and nontransgenic littermates, failed to transfer disease to BALB/c nude mice, suggesting tolerance induction within the thymus. We conclude that an immune response to the β subunit of the H/K ATPase complex initiates autoimmune gastritis. In addition, an autoimmune disease in which there are multiple autoantibody reactivities has been abrogated by the thymic expression of a single autoantigen.

Materials and Methods

Mice. BALB/c, C57BL/6, and Swiss mice for the production of transgenic mice were obtained from the Animal Resource Centre (Perth, Australia) or produced in the Monash University Medical School animal facility. Adult BALB/c mice for other experimental procedures were obtained from the Monash University Animal Services Centre (Melbourne, Australia). Progeny of transgenic mice were produced and housed under standard conditions at the Monash University Medical School animal facility. Transgenic mice for neonatal thymectomy were derived from lines 57 and 59 by mating the founder mouse with BALB/c mice, and from lines 25 and 50 by backcrossing the founder line twice to BALB/c mice. BALB/c nude mice used in thymocyte transfer experiments were obtained from the Animal Resource Centre. Thymocyte donors were derived from line 25 by backcrossing the founder line three times to BALB/c mice.

DNA Construction. The MHC class II I-E/H/K ATPase β subunit transgene (Fig. 1 *a*) was constructed as follows. A mouse H/K ATPase β subunit mini-gene was constructed by replacing a 960-bp BstEII fragment from a β subunit cDNA with a 3.97-kb BstEII fragment from a β subunit genomic clone (29). A 1.92-kb fragment encoding 5′ flanking sequence nucleotides −39 to −1964 of the MHC class II I-$E^k\alpha$ gene was blunt-end ligated to the 5′ end of the β subunit mini-gene. A 350-bp fragment encoding the SV40 small t intron splice site and polyadenylation signal was ligated 3′ of the mini-gene. The 5.9-kb construct was isolated from pBluescript SK- (Stratagene Inc., La Jolla, CA) by NotI and XhoI restriction enzyme digestion.

Generation of βH/K-transgenic Mice and Screening. βH/K-transgenic mice were generated using techniques previously described (30). The 5.9-kb transgene encoding the MHC class II I-E promoter and H/K ATPase β subunit mini-gene was purified on a nucleic acid chromatography system 52 column (BRL Life Technologies, Inc., Gaithersburg, MD), microinjected into the pronuclei of (BALB/c × C57BL/6)F_1 × BALB/c eggs, and transferred into the oviducts of pseudopregnant Swiss mice according to the method of Hogan et al. (31). βH/K-transgenic mice were identified by PCR analyses of mouse tail genomic DNA (31). PCR oligonucleotide primers designed to specifically amplify a region of 700 bp spanning the I-E promoter and β subunit mini-gene were 5′-CCCTTGAAAGCAGTCTTCCC-3′ and 5′-GTGCAGGGTGTGTGTGAG-3′, respectively. PCR was performed in 50-μl reactions containing amplification buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 0.1% gelatin), 200 μM of all four deoxynucleotides, 50 pmol oligonucleotide primers, and 2.5 U Taq DNA polymerase (Perkin Elmer Cetus, Branchbury, NJ). 30 cycles of 1.5 min at 95°C, 1.5 min at 65°C, and 3 min at 72°C were performed in a cycling waterbath. 10-μl samples were subjected to agarose gel electrophoresis, transferred to Nylon Hybond-N^+ membrane (Amersham International, Buckinghamshire, England), incubated with prehybridization solution (6× SSC, 7% SDS, 0.1% skim milk powder) (1× SSC is 0.15 M NaCl/0.14 M tri-sodium citrate) at 65°C for 1–2 h, and hybridized overnight with ^{32}P-labeled probes in prehybridization solution. Membranes were washed at 65°C in 0.1× SSC/0.5% SDS and fluorographed using Cronex intensifying screens (DuPont Co., Wilmington, DE) and x-ray film (Fuji) at −70°C. Four βH/K-transgenic lines (lines 25, 50, 57, and 59) were generated for analysis in this study.

mRNA Analysis. Transgene expression was detected by PCR analysis of cDNA generated from RNA. RNA isolation, reverse transcription, and PCR were performed essentially as previously described (32). Briefly, 3 μg of total RNA was reverse transcribed (Moloney murine leukemia virus reverse transcriptase; BRL Life Technologies Inc., Gaithersburg, MD) using oligo(dT) primer. The reaction mixture was divided in two and subjected to PCR using primers designed to amplify the H/K ATPase β subunit or actin cDNA. β subunit primers, 5′-GCAGGAGAAGAAGTCATGC-3′ and 5′-GGCTTTTGGGGATCATC-3′, generated a product of 617 bp, and actin primers (33), 5′-ATGGATGACGATATCGCTG-3′ and 5′-ATGAGGTAGTCTGTCAGGT-3′, generated a product of 568 bp. 30 cycles of 1.5 min at 95°C, 1.5 min at 60°C, and 3 min at 72°C were performed. 15-μl samples were subjected to agarose gel electrophoresis, transferred to Nylon Hybond-N^+ membrane, and hybridized with ^{32}P-labeled probes, washed, and autoradiographed as described above.

Neonatal Thymectomy. Transgenic mice from lines 25, 50, 57, and 59 were thymectomized on day 3 (day of birth designated as day 0) under cold anesthesia. The sternum was opened at the midline and after exposing the thymus, each lobe was aspirated with a Pasteur pipette attached to a vacuum. Mice were warmed with an infra-red heat lamp and returned to their mother. Incompletely thymectomized mice (6/117) were identified when killed and not analyzed further.

H/K ATPase ELISA. Anti-H/K ATPase α and β subunit antibodies in mouse sera were detected by ELISA essentially as previ-

ously described (34). Purified porcine H/K ATPase, comprising both α and β subunits, was purified by tomato-lectin affinity chromatography (35) and purity was established by SDS-PAGE and silver staining. Briefly, 96-well polyvinyl chloride round-bottomed microtiter plates (Dynatech Laboratories Inc., Chantilly, VA) were coated with 100 μl of purified H/K ATPase at a concentration of 0.5 μg/ml in 50 mM carbonate-bicarbonate buffer, pH 9.6. Plates were incubated with 1% BSA in PBS overnight at 4°C. Serum samples were analyzed at a dilution of 1:50 in PBS, 0.1% BSA. Bound antibody was detected by incubations of 100 μl biotinylated-rabbit anti-mouse Ig (Amersham International) diluted 1:500 in PBS, 0.1% BSA followed by 100 μl streptavidin-biotinylated horseradish peroxidase complex (Amersham International) at a dilution of 1:500 in PBS, 0.1% BSA. All incubations were performed at room temperature for 60 min. Between incubations, plates were washed three times with PBS, 0.05% Tween-20. Horseradish peroxidase activity was detected in each well with 100 μl substrate containing 0.2 mg/ml O-phenyldiamine (Sigma Chemical Co., St. Louis, MO) and 0.006% H_2O_2 for 30 min in the dark. The reaction was stopped with 50 μl 5 M H_2SO_4 and the reaction product was measured spectrophotometrically at 490 nm. Nonspecific reactivity for each serum was determined by assay of wells that did not contain H/K ATPase and were subtracted from the final optical readings. Inter-assay variation was corrected by reference to a standard of purified porcine H/K ATPase reacted with mAb 1H9, which is directed against the α subunit of the H/K ATPase (25).

Indirect Immunofluoresence. Anti-parietal cell autoantibodies were detected by indirect immunofluorescence on 5-μm sections of freshly frozen or paraffin-embedded normal mouse stomach (29). Sections of stomach were incubated with mouse sera at a dilution of 1:10 in PBS for 30 min at room temperature, followed by three washes with PBS. Bound antibody was detected by incubation with FITC-sheep anti-mouse Ig (Silenus Laboratories, Hawthorn, Victoria, Australia) at 1:50 in PBS, for 30 min at room temperature. After a further three washes with PBS, sections were mounted with Fluorsave Reagent (Calbiochem Behring Corp., San Diego, CA) and examined by epifluorescence microscopy with an excitation wavelength of 440–500 nm. mAbs 1H9 and 2B6, specific for the α and β subunits of the H/K ATPase, respectively, and sera from normal BALB/c mice were used as control antibodies (25).

Histology. Stomachs and ovaries were fixed in 10% formalin in phosphate buffer and embedded in paraffin. Sections (5 μm) were cut and stained with haematoxylin and eosin. Gastritis was assessed by the presence of cellular infiltrates into the gastric glands and muscularis mucosa (22). Oophoritis was assessed by ovarian atrophy and cellular infiltrates throughout the ovary and into ovarian follicles (22).

Thymocyte Transfer. Single-cell suspensions of thymocytes from 6–8-wk-old normal BALB/c mice, βH/K-transgenic, and nontransgenic littermates were prepared as previously described (36). Thymocytes (4×10^7) in 100 μl PBS were injected into the tail vein of 6–8-wk-old BALB/c nude mice. At 3 mo after transfer, mice were killed and anti-H/K ATPase antibodies and gastritis determined as described above. Mice from line 25 were used as thymocyte donors and were derived by backcrossing the founder line three times to BALB/c mice.

Results

Generation of βH/K-transgenic Mice and Gene Expression. The aim of this study was to examine the role of autoreactive T cells directed against the gastric H/K ATPase in the induction of autoimmune gastritis. Antigen expression in MHC class II–positive cells has been shown to cause antigen-specific clonal deletion within the thymus (37). Therefore, to eliminate potentially autoreactive H/K ATPase β subunit–specific T cells, we expressed the β subunit of the gastric-specific H/K ATPase within the thymus under the control of the MHC class II I-E^k_α promoter. The MHC class II I-E/H/K ATPase β subunit transgene (Fig 1 *a*) was injected into (BALB/c × C57BL/6)F_1 × BALB/c oocytes, and four lines (lines 25, 50, 57, and 59) of transgenic mice were generated. PCR analysis of RNA demonstrated that expression of H/K ATPase

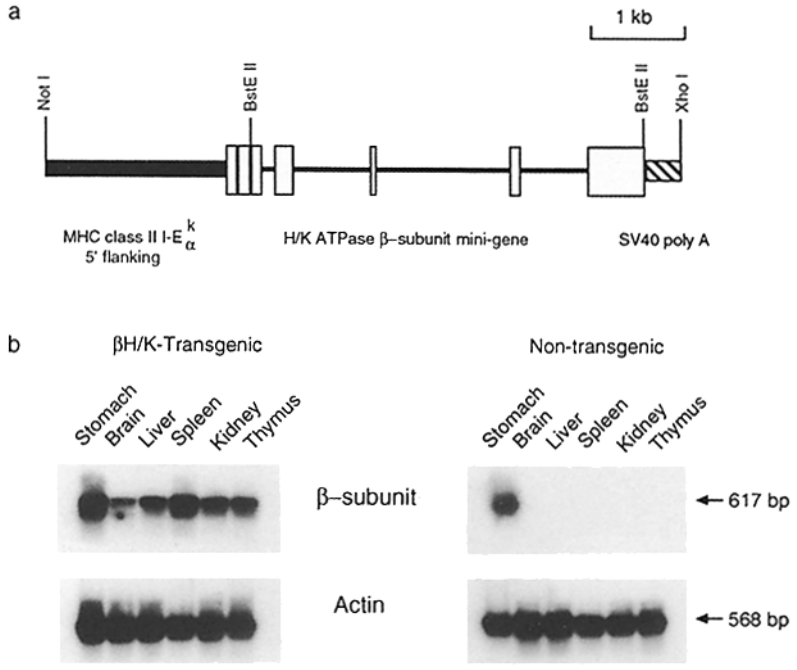

Figure 1. Structure and expression of H/K ATPase β subunit transgene. (*a*) Hybrid MHC class II I-E^k_α/H,K ATPase β subunit construct comprised the 5′ flanking sequence of the MHC class II I-E^k_α gene (*thick black line*), mouse H/K ATPase β subunit mini-gene, and a SV40 small t intron splice site and polyadenylation signal (*striped box*). The mini-gene comprised a cDNA fragment encoding exons 1 and 2 and a genomic fragment encoding exons 3–7 (exons: *open boxes*) and introns 3–6 (*thin black lines*). (*b*) PCR analysis of H/K ATPase β subunit and actin mRNA. RNA was isolated from line 25 transgenic mice and nontransgenic littermates from the tissues indicated. RNA was reverse transcribed using an oligo(dT) primer and amplified with oligonucleotides specific for the β subunit of the H/K ATPase (expected product size of 617 bp) and actin (expected product size of 568 bp) as indicated. PCR products were subjected to electrophoresis on a 1% agarose gel and then transferred to a nylon membrane. Products were detected by hybridization with appropriate ^{32}P-labeled probes. Bound probe was detected by fluorography. Analysis of actin mRNA served as an internal control and confirmed that similar amounts of RNA were used in each reaction.

β subunit mRNA in nontransgenic littermates occurred only in the stomach (Fig. 1 *b*). Prolonged exposure of fluorographs did not reveal expression in the thymus or any other nongastric tissue (data not shown). In contrast, βH/K-transgenic mice expressed H/K ATPase β subunit mRNA in all tissues examined, including the thymus (Fig. 1 *b*). Indirect immunofluorescence and immunoblotting of nonstomach tissues from βH/K-transgenic mice failed to detect H/K ATPase β subunit protein (data not shown). This was likely due to the rapid degradation of the β subunit (van Driel, I., unpublished data), and is similar to the findings with the α and β subunits of the related Na/K ATPase, which show instability when expressed as individual molecules (38).

Prevention of Experimental Autoimmune Gastritis. Littermates from line 25 were thymectomized at day 3 and assessed for the development of anti-H/K ATPase antibodies, gastritis, or oophoritis over a period of 5 mo. None (0/23) of the βH/K-transgenic mice developed antibodies to the α and β subunits of the H/K ATPase, parietal cell autoantibodies, or mononuclear cell infiltrates in the gastric mucosa (Fig. 2, *a* and *b*). A delayed immune response was excluded by the absence of anti-H/K ATPase antibody reactivity to up to 5 mo after thymectomy (Fig. 2 *a*). In our experience, maximal antibody reactivity is usually obtained by 3 mo postthymectomy (Alderuccio, F., personal observations). In contrast, thymectomized nontransgenic littermates developed anti-H/K ATPase antibodies with a frequency of 45% (9/20) (Fig. 2 *a*), an incidence consistent with previous reports (20). Mice were assessed for gastric mononuclear cell infiltrates by histological examination of stomachs (Fig. 3). All mice with H/K ATPase and parietal cell antibodies had cellular infiltrates in the gastric mucosa (Figs. 2 *b* and 3, *a* and *b*).

The occurrence of autoimmune oophoritis in neonatally thymectomized female BALB/c mice served as an internal control for our experimental system. Oophoritis was assessed by histological examination of ovaries (Fig. 3). The incidence of oophoritis in βH/K-transgenic (4/14, 28%) and nontransgenic (3/12, 25%) mice (Figs. 2 *b* and 3, *c* and *d*) was similar and consistent with previous reports (20). This indicated that βH/K-transgenic mice were still capable of initiating an autoimmune response to ovarian antigens, and that abrogation of autoimmune disease was gastric specific.

Anti-H/K ATPase antibodies were not found in transgenic littermates of three other independent βH/K-transgenic lines (lines 50, 57, and 59) (Fig. 4) after neonatal thymectomy. From the detailed analysis of transgenic line 25 and previously unpublished observations (Alderuccio, F., and T. Martinelli) the occurrence of anti-H/K ATPase antibodies is always accompanied by cellular infiltration of the gastric mucosa. Therefore, these data indicate that autoimmune gastritis was also prevented in these βH/K-transgenic lines. Furthermore, disease prevention was associated with transgene expression and not to chance insertion of the transgene into the mouse genome. Overall, in all four βH/K-transgenic lines, none (0/55) of the transgenic mice developed anti-H/K ATPase antibodies compared with 42% (24/56) of the nontransgenic littermates.

Tolerance Induction of Autoreactive T Cells within the Thymus. The prevention of experimental autoimmune gastritis in βH/K-transgenic mice after neonatal thymectomy suggests that the autoreactive T cells that cause disease in normal BALB/c or nontransgenic littermates have been tolerized. Since expression of the βH/K transgene was not confined to the thymus, it was not clear if T cell tolerance had occurred within the thymus or in the periphery. Thymocytes transferred from adult BALB/c mice to T cell–deficient BALB/c nude mice are capable of inducing autoimmune gastritis (14, 15). To assess the presence of autoreactive T cells within the thymus of βH/K-transgenic and nontransgenic littermates, thymocytes were transferred to BALB/c nude recipients. Autoimmune gastritis was induced in BALB/c nude mice by thymocyte transfer from normal BALB/c mice (5/6) and nontransgenic littermates (5/7), but not from βH/K-transgenic mice (0/7) (Fig. 5), suggesting that tolerance of pathogenic autoreactive T cells in the βH/K-transgenic mice was induced within the thymus.

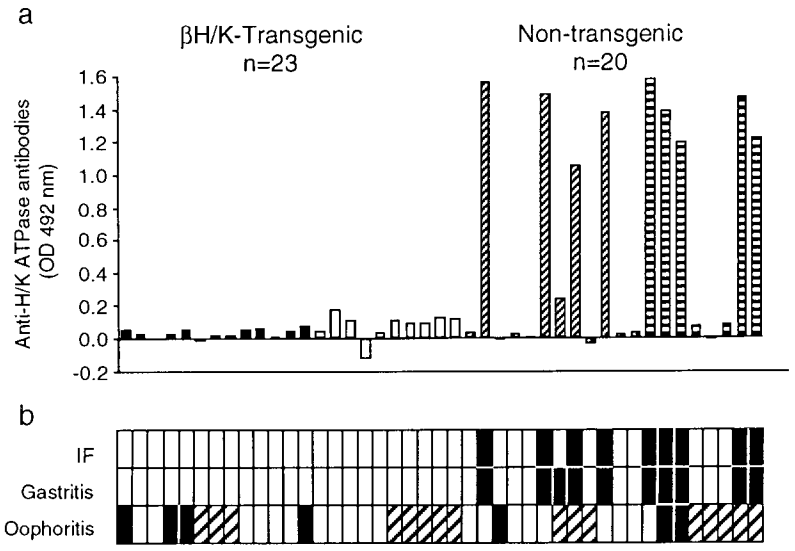

Figure 2. H/K ATPase autoantibodies, gastritis, and oophoritis in neonatally thymectomized mice. (*a*) Mice from line 25 were thymectomized at day 3 and transgenic status was determined at weaning. At 3 mo, sera were collected from all mice and approximately half the βH/K-transgenic and nontransgenic mice were killed for histological examination. Sera were collected from the remaining mice at 5 mo of age. Anti-H/K ATPase antibodies were detected by ELISA. *Filled bars*, βH/K-transgenic mice at 3 mo; *open bars*, βH/K-transgenic mice at 5 mo; *diagonal striped bars*, nontransgenic mice at 3 mo; *horizontal striped bars*, nontransgenic mice at 5 mo. (*b*) Immunofluorescence (*IF*) to detect anti–parietal cell autoantibodies and histological examination of stomachs and ovaries of mice from *a*. Mice are represented in the same order as *a* and are shown directly below the corresponding ELISA readings. The presence of parietal cell autoantibodies, gastritis, or oophoritis is indicated by a *filled box*. Striped boxes indicate male mice excluded from analysis of oophoritis.

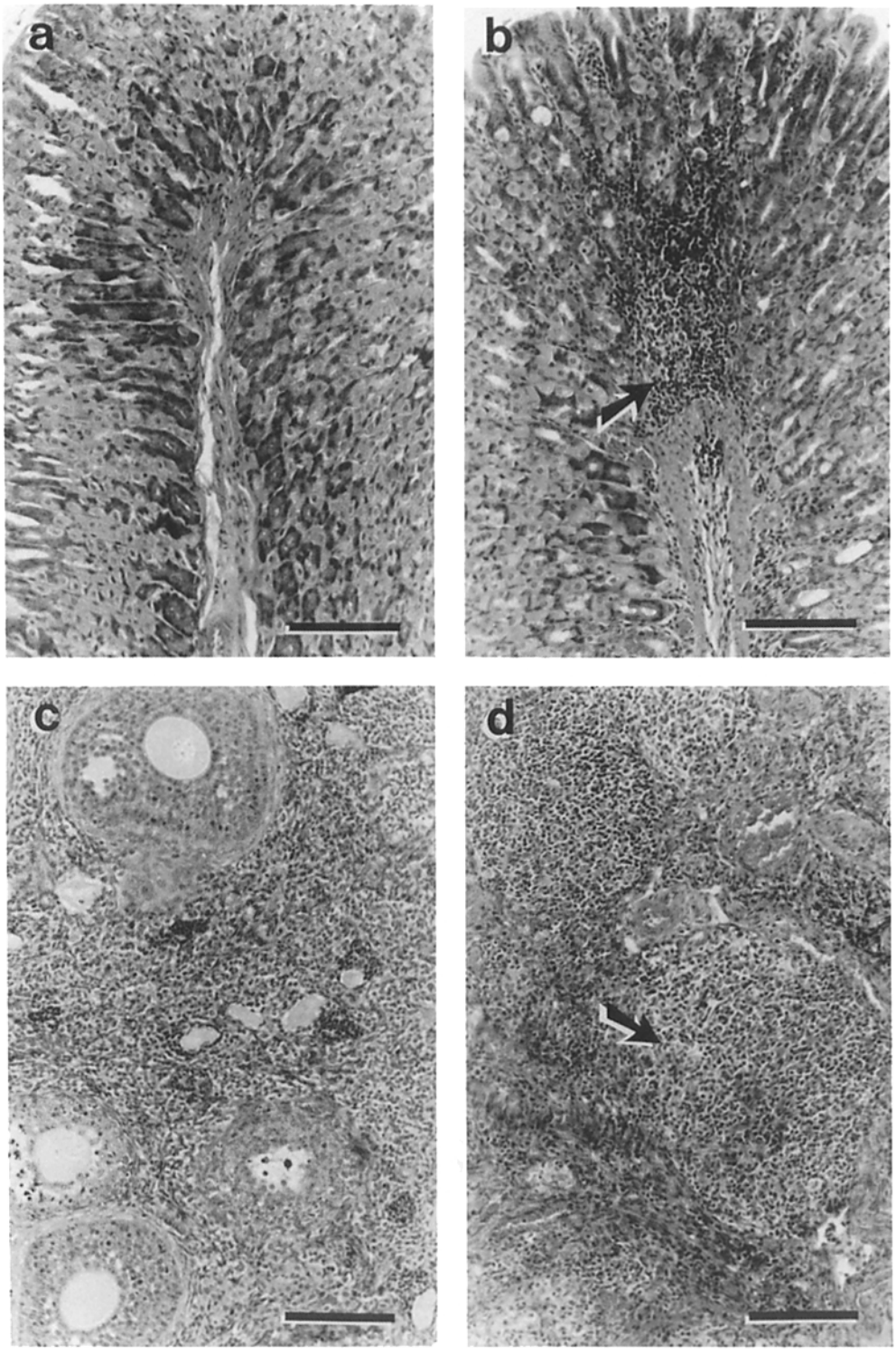

Figure 3. Histopathology of autoimmune gastritis and oophoritis after neonatal thymectomy. Mice from line 25 were thymectomized on day 3 and killed 3 mo later. Stomachs and ovaries were removed and fixed, sectioned, and stained with haematoxylin and eosin. (*a*) Stomach of transgenic littermate showing normal gastric morphology. (*b*) Stomach of nontransgenic littermate. (*Arrow*) Prominent mononuclear cell infiltrate. (*c*) Ovary of nontransgenic littermate displaying the normal appearance of healthy ovary containing numerous maturing ovarian follicles. (*d*) Ovary of transgenic littermate. Atrophy of ovarian structure and infiltration of follicles with numerous mononuclear cells (*arrow*) is apparent. Bars, 50 μm.

Discussion

The pathogenesis of many autoimmune diseases relies upon autoreactive T cells (10, 11, 16, 39), however, specificities of the T cells that induce autoimmune diseases are not known. Many investigations into autoimmunity have concentrated on the autoantibody response apparent in the later stages of disease when the immune system is recognizing several target molecules. However, it is unclear whether these end-stage responses are of relevance to the initiation of the disease process. An initial immune response with accompanying tissue destruction and inflammation will result in the influx of lymphocytes with multiple antigen specificities. The release of

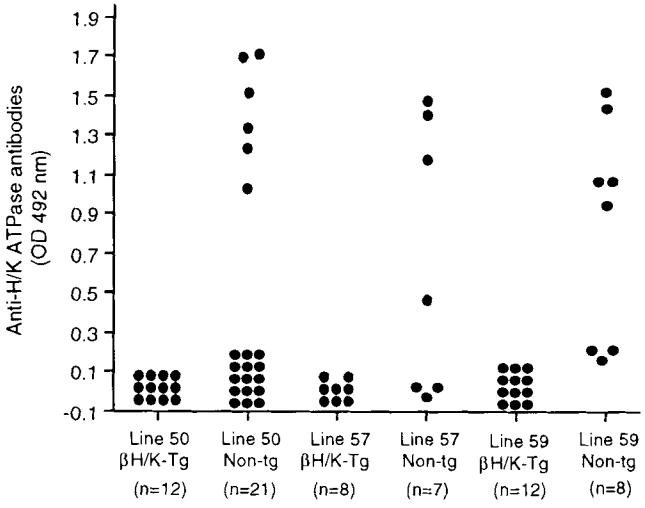

Figure 4. Anti-H/K ATPase antibodies in βH/K-transgenic (*βH/K-Tg*) and nontransgenic (*Non-tg*) mice from lines 50, 57, and 59. Mice were thymectomized at day 3. Transgenic status and the presence of anti-H/K ATPase antibodies were determined at 3 mo of age. Anti-H/K ATPase antibodies were detected by ELISA. Optical density readings >0.3 U were considered positive.

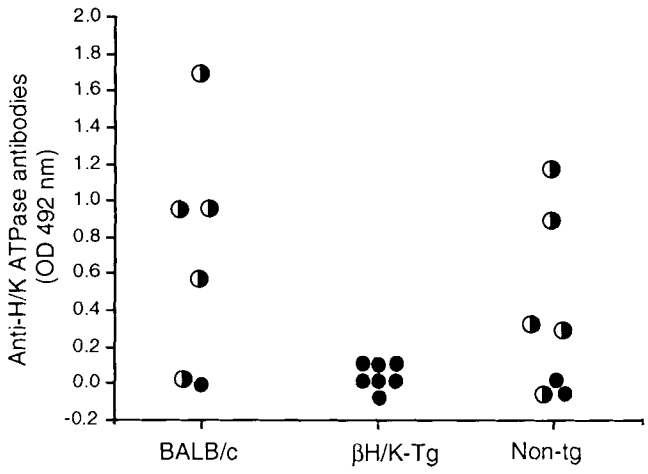

Figure 5. Incidence of anti-H/K ATPase antibodies and autoimmune gastritis in T cell–deficient BALB/c nude mice after thymocyte transfer. Thymocytes from normal BALB/c mice, line 25 βH/K-transgenic mice (*βH/K-Tg*), or nontransgenic littermates (*Non-tg*) were transferred to BALB/c nude mice. After 3 mo mice were killed and anti-H/K ATPase antibodies were detected by ELISA, and stomachs were examined for mononuclear cell infiltrates. Mice with gastritis are represented by a half-filled circle.

sequested antigens may initiate a secondary immune response that could become dominant. Hence, it can not be assumed that autoantigens detected late in disease are involved in the initiation of autoimmunity. The presence of lymphocytic infiltrates in the gastric mucosa in human autoimmune gastritis (17) and the central role of $CD4^+$ T cells in thymectomy-induced autoimmune gastritis (14, 15) suggest that autoreactive T cells are involved in the pathogenesis of this disease. Previous investigations have demonstrated that the sera of mice and humans with autoimmune gastritis contain antibodies directed to the α and β subunits of the gastric H/K ATPase (24–27). This study has investigated the possible role of H/K ATPase β subunit–specific T cells in the development of autoimmune gastritis.

Clonal deletion of developing T cells within the thymus can be directed by MHC class II–positive cells (37). Four lines of transgenic mice were produced in which expression of the gastric H/K ATPase β subunit was driven by the 5′ flanking region of the MHC class II $I\text{-}E^k_\alpha$ gene (40). H/K ATPase β subunit mRNA expression was found in all tissues examined from βH/K-transgenic mice, including the thymus. We reasoned that during T cell development, autoreactive T cells specific for the H/K ATPase β subunit would be tolerized within the thymuses of βH/K-transgenic mice. Thus, autoimmune gastritis would be prevented if T cells of this specificity are required for the onset of disease.

We neonatally thymectomized mice from four independent βH/K transgenic mouse lines. Transgenic littermates failed to develop anti–parietal cell autoantibodies and mononuclear cell infiltrates in the gastric mucosa. In contrast, the incidence of autoimmune gastritis in thymectomized nontransgenic littermates was at the expected frequency, as defined by the presence of cellular infiltrates in the gastric mucosa. The same pattern of disease prevention was found in all the four independent βH/K-transgenic lines, indicating that the phenomenon observed was reproducible and not associated with the insertion of the βH/K transgene within a particular genetic locus. Neonatally thymectomized BALB/c mice also develop oophoritis (20). The incidences of oophoritis in both βH/K-transgenic mice and nontransgenic littermates were identical. This finding indicated that the immune system of transgenic mice was still capable of initiating an autoimmune response to ovarian antigens. Thus the prevention of thymectomy-induced autoimmune disease in β-H/K-transgenic mice was gastric specific. From these data we conclude that a T cell response to the H/K ATPase β subunit is an absolute requirement for the development of autoimmune gastritis. Furthermore, it appears that the immune response to the other molecular target in this disease, the H/K ATPase α subunit, is either insufficient to cause disease, or is a secondary event to an anti-β subunit response.

In contrast to thymocytes from normal animals, thymocytes from βH/K-transgenic mice were unable to induce autoimmune gastritis when transferred to nude mice. This suggests that in βH/K transgenic mice disease-causing T cells are being silenced within the thymus. This finding has general implications for tolerance to peripheral self-antigens. It suggests that tolerance to the H/K ATPase β subunit in normal animals must be occurring at an extra-thymic location. However, our data do not directly address whether the mechanism of tolerance in the periphery is deletion, anergy, or suppression (41–43). It has been proposed that tolerance to extra-thymic antigens may involve transport of antigen to the thymus by recirculating macrophages (44). An implication of our findings is that the gastric antigen either does not reach the thymus, or does not accumulate at a concentration sufficient to cause thymic tolerance.

The prevention of autoimmune gastritis in βH/K-

transgenic mice after neonatal thymectomy was accompanied by the absence of autoantibodies not only to the β subunit of the H/K ATPase, but also to the α subunit. One explanation for the absence of α subunit immune response is that the α subunit does not contain MHC class II–restricted T cell epitopes. In which case, the B cell response to the α subunit of the H/K ATPase may be driven by β subunit–specific T cell help. This suggestion is similar to the intrastructural help described by Goodman-Snitkoff et al. (45), in which T cell help induced by an epitope on one molecule could drive a B cell response to a second unrelated, but physically associated, molecule. In many autoimmune diseases, the autoantigens that drive the autoimmune response are associated in subcellular organelles or subcellular particles, such as the nucleosome in systemic lupus erythematosus and the nucleolus in scleroderma (2, 8). Thus, if monospecific T cells can drive multiple B cell responses to closely associated molecules such as autoantigens in subcellular particles, it may explain the autoantibody profiles seen in many autoimmune diseases.

Alternatively, an immune response to the α subunit may require an initial immune response to the β subunit resulting in tissue damage and inflammation. The α subunit–specific T and B cell immune responses associated with autoimmune gastritis may be secondary phenomena not associated with initiation of disease. This possibility is similar to the model of "determinant spreading" described recently by Lehmann et al. (46). The α subunit may carry "cryptic" determinants (46) that become immunogenic after the initial immune response to the β subunit. Thus the role of the α subunit in the induction of autoimmune gastritis is still to be resolved. We will elucidate the role of the H/K ATPase α subunit in experimental autoimmune gastritis by the generation of α subunit transgenic mice in which the H/K ATPase α subunit will be expressed within the thymus.

Our findings here argue that an immune response to the β subunit of the gastric H/K ATPase is essential for the development of autoimmune gastritis. The ability to identify the causative antigens in autoimmune diseases has obvious ramifications in developing successful immunotherapeutic strategies. This approach, directed towards the identification of T cell targets in autoimmune gastritis, is applicable to other autoimmune diseases and will help to expand our understanding of the mechanisms and molecular basis of autoimmune diseases.

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