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Local Transgenic Expression of Granulocyte Macrophage-Colony Stimulating Factor Initiates Autoimmunity

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Mechanisms leading to breakdown of immunological tolerance and initiation of autoimmunity are poorly understood. Experimental autoimmune gastritis is a paradigm of organ-specific autoimmunity arising from a pathogenic autoimmune response to gastric H/K ATPase. The gastritis is accompanied by autoantibodies to the gastric H/K ATPase. The best characterized model of experimental autoimmune gastritis requires neonatal thymectomy. This procedure disrupts the immune repertoire, limiting its usefulness in understanding how autoimmunity arises in animals with intact immune systems. Here we tested whether local production of GM-CSF, a pro-inflammatory cytokine, is sufficient to break tolerance and initiate autoimmunity. We generated transgenic mice expressing GM-CSF in the stomach. These transgenic mice spontaneously developed gastritis with an incidence of about 80% after six backcrosses to gastritis-susceptible BALB/c mice. The gastritis is accompanied by a chronic inflammatory infiltrate in the gastric mucosa with loss of parietal and zymogenic cells. It is also associated with autoantibodies to the gastric H/K ATPase. The mechanisms of disease induction in mouse models of EAG are unknown. A role has been suggested for regulatory T cells in maintaining tolerance to autoantigens such as the gastric H/K ATPase (17–21). Sakaguchi and colleagues (18) have proposed that day 3 thymectomy prevents seeding to the periphery of thymic-derived CD4+CD25+ regulatory T cells. They have shown that normal splenocytes depleted of CD4+CD25+ T cells induce autoimmune gastritis when transferred to nude (nu/nu) mice (17, 22). Conversely, adoptive transfer of these regulatory T cells prevented autoimmunity induced by neonatal thymectomy or by adoptive transfer of pathogenic T cells (18). EAG induced by lymphopenia requires drastic manipulation of the immune system such as neonatal thymectomy. This limits their usefulness in studies aimed at understanding how tolerance in an intact immune system can be broken to initiate autoimmunity.

Our study was designed to address whether tolerance can be broken and gastric autoimmunity can be initiated in mice with an intact immune system by local expression of the pro-inflammatory cytokine GM-CSF in the stomach. We selected this cytokine for local tissue expression because we have previously identified GM-CSF in gastric lesions of mice with EAG (11) and because GM-CSF is a key cytokine required for proliferation and differentiation of not only granulocytes and macrophages but also of dendritic cells (23). Vaccination with GM-CSF strongly augments the immune response (24, 25). Too much GM-CSF can be lethal, because systemic tg expression of GM-CSF results in excessive accumulation and activation of granulocytes and macrophages (26, 27). Local GM-CSF expression has been shown to induce local inflammatory responses. For instance, mice infected with GM-CSF-expressing adenovirus. 

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3 Abbreviations used in this paper: EAG, experimental autoimmune gastritis; tg, transgenic; PC-GMCSF tg, parietal cell-GM-CSF tg; nu/nu, nude.

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have infiltrates of granulocytes and mononuclear cells in the lung (28). Tg expression of GM-CSF in GM-CSF deficient mice (29) corrects alveolar proteinosis associated with the deficient mice (30). Mice deficient in GM-CSF have a marked reduction in incidence and pathology of collagen-induced arthritis (31), while administration of GM-CSF accelerates the onset and pathology of arthritis (32).

Here we report that local expression of GM-CSF in the stomach of gastritis-susceptible BALB/cCrSlc mice results in development of autoimmune gastritis associated with circulating parietal cell autoantibodies to the gastric H/K ATPase. These characteristics are identical with those of autoimmune gastritis induced by neonatal thymectomy, immunization, altered T cell repertoire, subthalamic irradiation, and spontaneously in C3H/He mice (5, 6, 33–35).

Materials and Methods

Mice

BALB/c and (BALB/c × C57BL/6)F1, mice used for tgf mice production and BALB/CrCrSlc were maintained at Monash University Medical School animal facilities (Victoria, Australia). PC-GM-CSF tg mice were backcrossed to BALB/CrCrSlc at least four times.

Parietal cell GM-CSF (PC-GMCSF) transgene construction

The transgene directing GM-CSF expression to parietal cells of the stomach was generated as follows. The gene encoding murine GM-CSF was isolated from pUCS (27) by BanHI and EcoRI restriction enzyme digestion to release a fragment of −2.6 kb. This included a 310-bp HaeIII 5’XH7 fragment that was originally cloned into the SphI site in the 3’ untranslated region (27). This fragment was subcloned into the SpeI site of pBluescript between 10.9 kb murine gastric H/K ATPase β-subunit 5’ untranslated region (36) and an SV40-derived polyadenylation signal. All fragments were blunt ended before ligation and orientation of the GM-CSF gene was determined by restriction fragment mapping. The 13.95-kb transgene was isolated by NotI and Xhol restriction enzyme digestion and purified on a nucleic acid chromatography system 52 column (Life Technologies, Gaithersburg, MD).

Generation of PC-GM-CSF tg mice

Isolated transgene was resuspended in 10 mM Tris-HCl (pH 8.0) and 1 mM EDTA, at a concentration of 2–5 µg/µl injected into pronuclei of fertilized (BALB/c × C57BL/6) × BALB/c oocytes and transferred to oviducts of pseudopregnant BALB/c mice according to the method of Hogan et al. (37). Tg founders were identified by PCR analysis of extracted mouse-tail DNA. Oligonucleotides 5’-CTT CAC ACA GAG GAG ACT A-3’ and 5’-ATGAGGTAGTCTGTCAGGT-3’ were designed to generate a 374-bp product from the mouse insulin gene. PCR was performed in 25-µl reaction volumes containing amplification buffer; 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 1.5 mM MgCl2; 0.1% gelatin; 200 µM each dATP, dCTP, dGTP and dTTP; 50 pmol oligonucleotide primers; and 1.5 U Taq DNA polymerase (Life Technologies, Melbourne, Australia). Reaction mixtures were incubated at 95°C for 2 min and 30 cycles of 92°C for 30 s, 60°C for 30 s, and 72°C for 1 min, with a final cycle at 72°C for 5 min. Fifteen-microliter samples of PCR product were separated by agarose gel electrophoresis and visualized using UV illumination. Images were captured by digital camera and inverted for publication.

Messenger RNA analysis

Transgene expression of GM-CSF mRNA was detected by RT-PCR essentially as previously described (13). Total RNA was isolated from lung, spleen, heart, liver, and stomach using the Ultraspec II RNA isolation kit (Biotecx Laboratories, Houston, TX). Briefly, 2 µg of total RNA was reverse transcribed with Moloney murine leukemia virus reverse transcriptase (Life Technologies, Gaithersburg, MD) using oligo(dT) primer in a total volume of 20 µl. Two microliters of the reaction mixture was subject to PCR using primers designed to amplify the tg GM-CSF or actin cDNA. Actin primers were 5’-GTTGTTTCTATGCTACATCTG-3’ and 5’-GACTGACGCGGTGCTTGTG-3’ and generated a product of 568 bp. Tg GM-CSF was detected following two rounds of PCR. The first-round primers were sense 5’-CTATAAGCCCTGAGCGGCGC-3’ and anti-sense 5’-CCT CAC ACA GAG GAG ACT A-3’

ELISA, indirect immunofluorescence, and flow cytometry

Circulating anti-H/K ATPase autoantibodies were assayed by ELISA on 96-well plates coated with purified pig H/K ATPase as previously described (13). Anti-parietal cell autoantibodies were detected by indirect immunofluorescence on frozen or paraffin-embedded sections of normal mouse stomach (13). Gastric H/K ATPase α- and β-subunit reactivity was detected by immunofluorescence reactivity with Sf9 cells infected with baculovirus encoding rat ATPase α- or β-subunit. Recombinant baculovirus was obtained from E. Shevach (National Institutes of Health, Bethesda, MD), mAbs 1H9 and 2B6, reactive with the gastric H/K ATPase α-subunit and β-subunit, respectively, were used as controls.

Histology

Tissues were fixed in 10% formalin in PBS and embedded in paraffin. Five-micrometer stomach sections were stained with hematoxylin and eosin and viewed by light microscopy. Gastritis was assessed by the presence of cellular infiltrate within the gastric mucosa. Destructive gastritis comprised the presence of cellular infiltrate within the gastric mucosa with destruction of the cells within gastric glands. Other tissues were also examined for the presence of pathology.

In vitro proliferation assay

Pooled single cell suspensions of lymphocytes from tg (n = 5) and non-tg (n = 6) littermates were prepared by gently grinding lymphoid tissues between frosted glass slides and used as responders in in vitro proliferation assays. Non-tg splenocytes were treated with ammonium chloride solution (0.9%) to lyse RBC and irradiated (3000 rad) for use as APCs. Cells were suspended in RPMI 1640 culture media supplemented with 10% FCS, 100 µm penicillin, 100 µm streptomycin, 2 mM L-glutamine, and 50 µM 2-ME. Proliferation assays were performed in 96-well tissue culture plates (Dynex Technologies, Chantilly, VA) in a total volume of 200 µl containing 2.5 × 105 responder cells, 2.5 × 104 irradiated APCs, and Ag. For in
vitro assay for CD4+CD25+ regulatory T cells, splenocytes were sorted into CD4+CD25+ and CD4+CD25- populations, with purity of 99% and 98%, respectively. Proliferation assay was performed in a total volume of 200 µl containing 2 × 10^5 responders, 5 × 10^5 irradiated APCs, and Ag. Con A was used at a final concentration of 3 µg/ml and IL-2 was used at 100 U/ml. Cells were incubated for 48 h at 37°C/10% CO_2 followed by an additional overnight incubation in the presence of 1 µCi [³H]thymidine (NEN, Boston, MA). Cells were harvested onto glass filters (Skatron, Sterling, VA) suspended in scintillant and [³H]thymidine incorporation was determined on a Wallac 1205 Betaplate liquid scintillation counter (Pharmacia, Uppsala, Sweden). Control wells were comprised of responder cells alone, APCs alone or proliferation in the absence of Ag.

Cell transfer studies

Single-cell suspensions were prepared from pooled spleens, paragastric lymph nodes, inguinal lymph nodes, and stomachs (41) in HBSS/1% FCS. Two groups of PC-GMCSF tg (n = 6 and n = 3) mice with circulating parietal cell and H/K ATPase Abs and one group of non-tg (n = 3) littermates were used in these experiments. Splenocytes were treated with ammonium chloride solution (0.9%) to lyse RBC. Cells to be injected were washed and resuspended in HBSS in a total volume of 150–200 µl. Cells were transferred to BALB/c nu/nu mice by i.v. tail vein injection. Recipient mice received 4 × 10^7 splenocytes, 1–2 × 10^7 inguinal lymph node cells, 1.3–2 × 10^7 paragastric lymph node cells, or 5 × 10^7 stomach infiltrate cells. Mice were killed at 8–12 wk following cell transfer and sera were analyzed for H/K ATPase and parietal cell autoantibodies. Stomachs and other tissues were processed for paraffin-embedded sections and examined by histology for gastritis.

For transfer of purified CD4+ and CD8+ T cells, pooled paragastric lymph node cells were isolated in two separate experiments from six and five PC-GMCSF tg mice with circulating parietal cell and H/K ATPase Abs. Cells were stained with anti-CD4-PE and anti-CD8-FITC and sorted using a FACS caliber cell sorter (Becton Dickinson). Analysis of sorted populations revealed a purity for CD4+ and CD8+ T cells of 97% and 98%, respectively. Each BALB/c nu/nu recipient received 1.5–2 × 10^6 cells by i.v. injection. Eight weeks following transfer, mice were killed and examined as described above.

Statistical analysis

Cell numbers and populations were compared using a two-tailed t test. Cell transfer results were compared using a Fisher’s exact test.
H/K ATPase expressed in insect Sf9 cells. Reactivity of anti-H/K ATPase reactive sera with parietal cells was confirmed by indirect immunofluorescence (Fig. 3B) in which the staining pattern was identical with that observed with mAbs to gastric H/K ATPase (Fig. 3C). We noted that three sera that reacted with baculoviral Ags did not react with parietal cells or H/K ATPase by ELISA. This may reflect differences in sensitivity of the assays or in the Ags used. It is difficult to draw any conclusions because it is not known whether the mice in question may have gone on to develop gastritis if left for a longer period of time. Mice that displayed parietal cell and H/K ATPase autoantibodies had morphological and histological evidence of destructive gastritis. The gastritis was characterized macroscopically by mucosal hypertrophy (Fig. 3E) accompanied by dramatic enlargement of draining para-gastric lymph nodes (not shown) and microscopically by submucosal mononuclear cell infiltrate that extended into the lamina propria with destruction of parietal and zymogenic cells (Figs. 2 and 3E). With further backcrossing of PC-GMCSF tg mice to BALB/cCrSlc mice, the incidence of gastritis increased to 57% (8/14) and 82% (9/11), respectively, in mice backcrossed five and six times. Non-tg mice did not develop circulating parietal cell autoantibodies or gastritis (Fig. 3, A and D). Histological examination of non-gastric tissues including, heart, kidney, liver, and pancreas indicated that the inflammatory cellular infiltrate was confined to the gastric mucosa of PC-GMCSF tg mice (data not shown).

Influx of dendritic cells and macrophages precedes CD4 T cells in the gastric lesion of PC-GMCSF tg mice

Frozen stomach sections from 4- and 8-wk-old PC-GMCSF tg mice with parietal and H/K ATPase autoantibodies and non-tg littermates were examined by confocal microscopy with Abs specific for CD4 T cells, CD8 T cells, B cells, macrophages, dendritic cells, and granulocytes (Fig. 4). Representative sections stained with hemotoxylin and eosin confirm hypertrophy and presence of mononuclear infiltrates within the gastric mucosa of PC-GMCSF tg mice (Fig. 4B) compared with non-tg littermates (Fig. 4A). Sections were double stained to visualize parietal cells and various cell surface markers specific for CD4+ T cells, CD8+ T cells, B cells (B220), dendritic cells (CD11c), macrophages (CD11b), and granulocytes (Gr1). Firstly, we found that stomach sections from 8-wk-old non-tg littermates were not completely void of leukocytes (Fig. 4) with occasional staining of cells observed in some sections. This is consistent with our earlier findings in nonthymectomized mice in which occasional macrophages and lymphocytes were also observed in the gastric mucosa (11). In contrast, there was a profound difference in the staining pattern observed with 8-wk-old PC-GMCSF tg mice. There was a dramatic influx of CD4 T cells (Fig. 4E), dendritic cells (Fig. 4K), macrophages (Fig. 4N), and granulocytes (Fig. 4Q). However, it should be noted that CD11b can also be found on dendritic cells and granulocytes, and thus the staining...
**FIGURE 4.** Immunohistochemistry of stomachs from PC-GMCSF tg mice. Five-micrometer frozen stomach sections were prepared from 8-wk-old non-tg (A, D, G, J, M, P, S) and PC-GMCSF tg mice (B, E, H, K, N, Q, T) and 2-wk-old PC-GMCSF tg mice (C, F, I, L, O, R, U). Sections were stained with hemotoxylin and eosin (A–C) to visualize stomach morphology and confirm the presence of gastritis in tg mice. Parietal cells (stained red) were identified using the lectin, *Dolichos biflorus*, which specifically binds to carbohydrates on parietal cells (D–R) or human sera with parietal cell reactivity (S–U). Fewer parietal cell staining in the 2-wk-old Tg mice is due to incomplete development of the gastric mucosa in young mice (36). FITC-conjugated Abs were used to identify cell surface markers: CD4 (D–F), CD8 (G–I), CD11c (J–L), CD11b (M–O) and Gr1 (P–R). B220 surface Ag was identified using a biotinylated-anti-B220 mAb followed by streptavidin-conjugated Texas Red (S–U). Fluorescence images were captured on Bio-Rad confocal microscope with identical exposure times. Sections from 8-wk-old mice were captured with a ×10 objective and 2-wk-old mice with a ×20 objective.
observed in Fig. 4N may not be entirely associated with macrophages. In some sections, B cells were observed in follicle-like aggregates (Fig. 4T) similar to that observed in the neonatal thymectomy model of EAG. CD8 T cells were not present (Fig. 4H), which is also similar to the neonatal thymectomy model (11, 41) in which CD8 T cells do not appear to be implicated in the pathogenesis of EAG (10). To identify early cellular events associated with the initiation of autoimmunity, we examined stomachs of 4- (with H/K ATPase reactivity) and 2-wk-old tg mice. Immunohistochemical analysis of the stomachs of 4-wk-old PC-GMCSF tg mice showed the presence of a heterogeneous cellular infiltrate similar to that observed in the 8-wk-old group except that B cell follicles were not observed (data not shown). This indicates that autoimmune gastritis in this model is well established by 4 wk of age and, therefore, accelerated compared with the neonatal thymectomy model in which only a minority of mice display evidence of disease at 4 wk of age (11). However, examination of 2-wk-old tg mice did reveal a difference in the composition of the cellular infiltrate. Of the leukocyte markers examined, staining was confined to cells isolated from the paragastric lymph node, or whether T cells isolated from other lymphoid organs can respond to gastric membranes. Cells were isolated from the spleen and from the paragastric, mesenteric, and inguinal lymph nodes. A proliferative response was observed only with T cells isolated from the paragastric lymph node (Fig. 5B). None of the cell preparations responded to liver membranes, included as controls (Fig. 5B). From these results it appears that PC-GMCSF T cells proliferate specifically to the H/K ATPase of gastric membranes and that the response is confined to cells from the paragastric lymph node.

**CD4+ T cells from PC-GMCSF tg mice transfer gastritis**

To determine whether PC-GMCSF tg mice can adoptively transfer gastritis, cells isolated from pooled tg and non-tg stomach, spleen, or paragastric or inguinal lymph nodes were transferred to BALB/c nu/nu mice in two separate experiments. Eight to 12 wk following transfer, recipient mice were killed, sera were assessed for H/K ATPase, and parietal cell autoantibodies and stomachs were examined for gastritis (Fig. 6). A destructive gastritis was observed in mice that received cells from tg spleen (1/2) (Fig. 6A), gastric lymph nodes (3/5), paragastric lymph nodes (2/5), and stomach (1/1); the gastritis was associated with circulating autoantibodies to parietal cells demonstrated by indirect immunofluorescence (Fig. 6B). These observations indicate that pathogenic lymphocytes are not confined to the paragastric lymph node or stomach, but were present in all lymphoid sources examined. Cells from non-tg spleen (n = 1), inguinal lymph node (n = 1), and paragastric lymph node (n = 1) did not induce gastritis in nu/nu mice (data not shown). This is consistent with previous observations that cells from normal mice do not transfer gastritis (9, 20, 21). To determine whether the pathogenic cells were CD4+ or CD8+ T cells, CD4+ and CD8+ cells from pooled paragastric lymph node were sorted by flow cytometry and transferred to syngeneic nu/nu mice. Pooled data from two experiments indicate that mice transferred with CD4+ T cells develop gastritis with circulating autoantibodies to parietal cells (5/6) while mice transferred with CD8+ T cells remained disease free (0/4, p = 0.048) (data not shown). The finding that CD4+ T cells transferred autoimmune gastritis is similar to that observed in EAG induced by neonatal thymectomy (10), in which CD4+ T cells have been shown to be the pathogenic T cells responsible for disease.
Lymphocyte and CD4\(^+\)CD25\(^+\) regulatory T cell populations are not perturbed in PC-GMCSF tg mice

The majority of mouse models of EAG result from an induced state of lymphopenia; be it neonatal thymectomy (43), repeated lymphoid irradiation (35), or tg skewing of the T cell repertoire (34). Therefore, total cell numbers and lymphocyte populations were analyzed in 6- to 8-wk-old PC-GMCSF tg to determine whether there were any differences in these parameters compared with non-tg mice. We found no difference in total number of cells recovered from the thymus (tg, \(n = 4\), 1.48 ± 0.5 \times 10^8; non-tg, \(n = 4\), 1.62 ± 1.83 \times 10^8; \(p = 0.89\)), spleen (tg, 1.79 ± 0.4 \times 10^9; non-tg, 1.85 ± 0.31 \times 10^9; \(p = 0.81\)), and inguinal lymph nodes (tg, 3.90 ± 2.74 \times 10^8; non-tg, 2.86 ± 1.59 \times 10^8; \(p = 0.54\)). Not unexpectedly, given the dramatic enlargement of the draining paragastri-chromic hypertropy observed in PC-GMCSF tg mice is similar to that property of these cells (20, 44). CD4\(^+\)CD25\(^+\) and CD4\(^+\)CD25\(^-\) cells were purified from pooled spleens of three gastritic PC-GMCSF tg mice. Although CD4\(^+\)CD25\(^+\) cells readily proliferated to Con A, CD4\(^+\)CD25\(^-\) regulatory cells were resistant to Con A stimulation (Fig. 7). Furthermore, CD4\(^+\)CD25\(^+\) regulatory cells inhibited proliferation of CD4\(^+\)CD25\(^-\) cells to Con A stimulation and this inhibition was abrogated by IL-2 (Fig. 7). These characteristics are similar to those previously described for this regulatory population (20, 44) and indicate that autoimmune gastritis in PC-GMCSF tg mice is not due to global perturbation of the CD4\(^+\)CD25\(^+\) regulatory population.

Discussion

EAG in mice is an animal model of human autoimmune gastritis, sharing the same target autoantigens, gastric pathology, and circulating autoantibodies to the \(\alpha\)- and \(\beta\)-subunits of the gastric H/K ATPase (1). The best characterized EAG models are those induced by lymphopenia (4). These models are of limited use for studies directed toward addressing how tolerance can be broken and autoimmunity initiated in animals with an intact immune system.

In this study, we generated tg mice that locally expressed GM-CSF in the stomach under the control of the gastric H/K ATPase \(\beta\)-subunit promoter (36). PC-GMCSF tg mice spontaneously developed characteristics of autoimmune gastritis with an incidence increasing from 40% in mice backcrossed four times to ~80% in mice backcrossed six times to gastritis-susceptible BALB/c/CrSlc mice. Circulating autoantibodies to gastric parietal cells were generated that reacted with the \(\alpha\)- and \(\beta\)-subunits of the gastric H/K ATPase. Stomachs from these PC-GMCSF tg mice with parietal cell Abs displayed an inflammatory infiltrate in the gastric mucosa. The infiltrates extended into the lamina propria with accompanying destruction of mucosal parietal and zymogenic cells. The gastritic hypertropy observed in PC-GMCSF tg mice is similar to that

![Image](http://www.jimmunol.org/)
described in other models of EAG (8, 35, 43) and has been attributed to the replacement of parietal and zymogenic cells with proliferating epithelial stem cells (7). Lymphoid cells recovered from the draining paragastric lymph nodes of PC-GMCSF tg mice specifically proliferated in response to stimulation with gastric Ags and purified H/K ATPase. CD4, but not CD8, T cells transferred gastritis to syngeneic nu/nu mice. These observations confirm the immunological nature of the gastritis. These characteristics are identical with the autoimmune gastritis observed in mice following neonatal thymectomy (13, 33, 43, 45), suggesting that the immunopathology in both models are similar.

GM-CSF is a pleiotropic cytokine that stimulates proliferation and maturation of macrophages and dendritic cells (23, 46, 47). In previous studies we have shown that GM-CSF is expressed in the gastric lesion of mice with EAG induced by neonatal thymectomy (11). A role for GM-CSF in autoimmune pathology has previously been suggested in collagen-induced arthritis in mice (31, 32). Although the exact mechanism by which GM-CSF expression induces autoimmune gastritis is not known, we suggest that local expression of GM-CSF transgene in the gastric mucosa has initiated an autoimmune response through activation of local APCs, most likely dendritic cells. In normal mice, immature APCs are implicated in the removal of apoptotic cells resulting from cellular turnover in the stomach (7); and in itself, this process would not be expected to result in activation of these APCs (48). In the presence of a pro-inflammatory cytokine such as GM-CSF, gastric APCs may be activated directly (49) or through the action of other induced cytokines such as TNF-α (50) and migrate to the local draining lymph node where they activate naive CD4+ T cells specific for the gastric H/K ATPase. The proposed effect of GM-CSF in enhancing dendritic cell activation and presentation has previously been exploited in designing vaccines against pathogens and tumors (24, 51, 52). The earlier influx of CD11c and CD11b reactive cells within the gastric mucosa of PC-GMCSF tg mice observed at 2 wk after birth preceding the influx of CD4 T cells observed at 4 wk supports a role for these APCs in the initiation of gastric autoimmunity. It is not known whether these cells are recruited to the gastric mucosa, arise from local proliferation or both. Similar findings have been observed following intramuscular injections with plasmids encoding GM-CSF (53). In these studies, expression of GM-CSF in muscle resulted in local accumulation of macrophages, dendritic cells and granulocytes but not CD4 T cells. The lack of CD4 T cells in the DNA vaccination study compared with our findings may reflect differences in the time span of the two experiments. The finding that an in vitro T cell proliferative response was only observed with T cells isolated from the local draining paragastric lymph node and not from other lymphoid organs supports the suggestion that activated APCs migrate to the draining lymph node to activate naive T cells homing to the lymph node. As with other models of autoimmune gastritis (8–10), the transfer and histochemical studies in this report suggest that CD4+ T cells are the pathogenic cells in EAG. Ag presentation and activation of T cells may also occur in the gastric mucosa, as has been suggested in animal models of diabetes (54). Certainly, we have observed organized lymphoid structures similar to those described by Ludewig and colleagues (54) in the gastric mucosa of mice with thymectomy-induced EAG (11) and also in the PC-GMCSF mice in the present study.

Local α expression of other pro-inflammatory cytokines such as TNF-α, IFN-γ, and IL-2 have previously been attempted to induce other models of organ-specific autoimmunity. For instance, expression of IL-2 or TNF-α in pancreatic islets of BL/6 mice resulted in insulitis without diabetes (55, 56), while in the NOD mouse, islet expression of TNF-α accelerated diabetes onset (50). These studies indicate that TNF-α expression in islets can promote local inflammation or aggravate diabetes in diabetes-prone mice. However, whether TNF-α can promote by itself a destructive autoimmune lesion is not clear. Although IFN-γ expression in pancreatic islets resulted in diabetes, it is unclear whether this is a consequence of autoimmunity or the result of local destruction induced by IFN-γ (57). In the present study, we show for the first time that expression of the pro-inflammatory cytokine, GM-CSF, in the gastric environment of gastritis-susceptible mice has induced autoimmune gastritis. This was confirmed by production of autoantibodies to the gastric H/K ATPase, a specific T cell response to the ATPase and transfer of disease by CD4 T lymphocytes from gastritis to nu/nu mice. These observations suggest that expression of GM-CSF in the local environment of the stomach is sufficient to break tolerance and initiate autoimmunity.

A defining feature of our study is that tolerance to gastric H/K ATPase has been broken without a major perturbation of the immune system, a feature associated with lymphopenic models of EAG (4, 58). EAG is not observed in normal BALB/c or BALB/cCrSlc mice. Experimental evidence suggests a role for regulatory CD4+CD25+ T cells in maintaining tolerance and that removal of this population from the normal repertoire renders the remaining lymphocytes pathogenic (17, 18). This is not the case in PC-GMCSF tg mice because we found CD4+CD25+ cells in the thymus and in the periphery of these mice. In vitro, we found that CD4+CD25+ T cells from the spleens of tg mice were anergic, could prohibit proliferation of CD4+CD25+ cells and their anergy could be reversed by exogenous IL-2. These properties are identical with those previously described for CD4+CD25+ regulatory cells (20, 44). Therefore, in PC-GMCSF tg mice, it appears that local production of GM-CSF in the stomach has initiated a pathogenic autoimmune response, and overcome suppressor activity of CD4+CD25+ regulatory T cells. Our observation that cells from the draining paragastric lymph node can be specifically stimulated to proliferate in the presence of gastric Ags supports this suggestion. The observation that autoimmune gastritis can be transferred from lymphoid sites other than the draining lymph node seems to contradict this. This apparent discrepancy may reflect the ability of circulating activated autoreactive T cells to expand following transfer to an “empty” periphery in nu/nu mice. In vitro, CD4+CD25+ regulatory cells are naturally anergic and do not proliferate in response to stimulation through the TCR. However, the anergy and suppressive activity of CD4+CD25+ T cells can be abrogated by IL-2 coupled with TCR stimulation (20, 44). Therefore, it is possible that in PC-GMCSF tg mice, local IL-2 generated by activated self-reactive CD4 T cells in the paragastric lymph node may have rendered CD4+CD25+ regulatory cells nonfunctional. The presence of this regulatory population may also explain why not all PC-GMCSF tg mice develop autoimmunity. However, with the incidence of gastritis approaching 100% with increasing numbers of backcrosses to gastritis-susceptible BALB/cCrSlc mice, local expression of GM-CSF alone may be sufficient to break tolerance and initiate autoimmunity in this mouse strain.

The PC-GMCSF tg mice generated in this study will permit us to address several important questions pertaining to the development of organ-specific autoimmunity. We have shown for the first time that local expression of a proinflammatory cytokine in the stomach of genetically susceptible mice can induce a damaging autoimmune response. Our observations support the recent findings of Sarvetnick and colleagues (59) that bystander damage resulting from responses to “danger” signals (60, 61) may be sufficient to initiate autoimmunity. Taken together, these observations support our proposed genesis of the gastric lesion in autoimmune gastritis initiated by the gastric H/K ATPase (62). The induction of
damaging autoimmunity in the presence of an intact immune sys-
tem will make this model useful for understanding mechanisms
associated with the breakdown of immune regulation in the local
lymph nodes leading to autoimmunity. Understanding this process
can be expected to lead to strategies designed to restore tolerance
and/or arrest progression of damaging autoimmunity.

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