Growth factors associated with gastric mucosal hypertrophy in autoimmune gastritis

Teo V. Franic,1 Louise M. Judd,2 Nhung V. Nguyen,1 Linda C. Samuelson,3 Kate L. Loveland,4 Andy S. Giraud,2 Paul A. Gleeson, and Ian R. van Driel1

1The Russell Grimwade School of Biochemistry and Molecular Biology, The University of Melbourne, Melbourne, VIC 3010; 2Department of Medicine, University of Melbourne, Western Hospital, Melbourne, VIC 3011; 3Monash Institute of Reproduction and Development and Australian Research Council Centre of Excellence in Biotechnology and Development, Monash University, Melbourne, VIC 3168, Australia; and 4Department of Molecular and Integrative Physiology, The University of Michigan, Ann Arbor, MI 48109–0622

Submitted 5 November 2003; accepted in final form 20 May 2004

Franic, Teo V., Louise M. Judd, Nhung V. Nguyen, Linda C. Samuelson, Kate L. Loveland, Andy S. Giraud, Paul A. Gleeson, and Ian R. van Driel. Growth factors associated with gastric mucosal hypertrophy in autoimmune gastritis. Am J Physiol Gastrointest Liver Physiol 287: G910–G918, 2004. First published June 17, 2004; 10.1152/ajpgi.00469.2003.—A prominent pathological feature of murine autoimmune gastritis is a pronounced mucosal hypertrophy. Here, we examined factors that may be responsible for inducing this hypertrophy. Because gastrin is known to be both an inducer of gastric mucosal cell proliferation and is elevated in autoimmune gastritis, mice deficient in gastrin were thymectomised at day 3 and assessed for autoimmune gastritis. Gastrin-deficient mice showed all the characteristic features of murine autoimmune gastritis, including gastric unit hypertrophy due to hyperproliferation and accumulation of immature epithelial cells, decreases in the number of zymogenic and parietal cells, and autoantibodies to the gastric H⁺/K⁺-ATPase. Hence, gastrin is not required for either the establishment of chronic gastritis or development of the typical pathological features of this disease. We also examined mRNA levels of a number of gastric mucosal growth factors in RNA samples from mice with hypertrophic autoimmune gastritis. Members of the Reg family, RegIIIβ and RegIIIγ, were greatly elevated in mice with hypertrophic gastritis, whereas RegI and amphiregulin (an EGF receptor ligand) were more modestly and/or inconsistently induced. These data demonstrate that induction of gastric mitogenic factors, such as members of the Reg family, can be achieved in inflammatory situations by gastrin-independent pathways. Members of the Reg family, in particular RegIIIβ and RegIIIγ, are good candidates to be involved in inducing the mucosal hyperproliferation in autoimmune gastritis. These findings are likely to be of relevance to other gastric inflammatory conditions. Reg genes; gastrin; gastric inflammation

Autoimmune gastritis is a disease mediated by CD4⁺ T lymphocytes directed to the subunits of the gastric H⁺/K⁺-ATPase and is one of the most common and thoroughly characterized autoimmune diseases (12, 27, 28, 30). The end-stage of this disease is pernicious anemia. Over 400,000 people are affected by pernicious anemia in the United States, and the estimated prevalence of autoimmune gastritis in Western populations over the age of 60 is 1.9% (27, 28). In certain strains of mice, thymectomy at day 3 results in a very high incidence of autoimmune gastritis (26). This disease in mice closely resembles the human disease, and much of the information on the immunopathological mechanisms of autoimmune gastritis has been gained from studying this model (30). Autoimmune gastritis is the result of the actions of CD4⁺ helper T lymphocytes directed to the gastric H⁺/K⁺-ATPase expressed by gastric parietal cells (28, 30). These CD4⁺ cells are both necessary and sufficient to cause disease (6).

The pathological features of autoimmune gastritis are more complex than a simple depletion of the parietal cells that are the direct cellular target of the T cell infiltrate. Rather, we have demonstrated that there is a disruption of the normal cellular homeostatic mechanisms in the gastric mucosa, resulting in the accumulation of elevated numbers of immature cells and thus pronounced epithelial hypertrophy (15). In addition, there is a block in production of parietal and zymogenic cells. At present, it is unclear what factors drive this breakdown in cellular homeostasis in gastric mucosa.

The units (glands) of the gastric mucosa contain populations of proliferating cells that are precursors of terminally differentiated cells. These self-renewing immature cells are required to generate end-stage cells during ontogenic development and to replace mature cells as they rapidly turn over in the units of adult animals (18). In addition, proliferative rates of immature cells are increased in damaged mucosa, presumably to aid in regeneration. A number of factors has been demonstrated to cause proliferation of immature gastric mucosal cells. Peptides encoded by the gastrin gene (gastrins) are potent stimulators of gastric mucosal cell proliferation (7). Gastrin levels are elevated in mice (21, 31) and humans (31) with autoimmune gastric disease and therefore may be involved in the hypertrophy observed in these diseases. Gastrins do not act by binding directly to proliferating immature cells of the gastric mucosa, because these cells do not express gastrin/CCCK receptors (7). Rather they probably induce enterochromaffin-like (ECL) cells and parietal cells to release factors that act on immature cells (7, 20). These hormones may include members of the EGF family, transforming growth factor-α (TGF-α), heparin-binding EGF (HB-EGF), and amphiregulin, which are secreted by parietal cells (2, 29), and the Reg proteins (11).

Reg proteins are a recently defined family of growth factors. To date, most work has focussed on the RegI molecule. RegI is expressed by ECL and zymogenic cells in the human gastric...
mucosa (14) but only by ECL cells in the rat (4). Fuikui et al. (11) demonstrated that RegI induces proliferation of gastric epithelial cells and plays a role in the proliferative response mediated by gastrin. RegI is also induced in ECL cells by the neutrophil-induced chemokine CINC-2β in stress-induced gastric injury (19). Other members of the Reg family, namely the RegIII subfamily, are also expressed in the gastric mucosa (1). At present, there is no information on the roles of RegIII proteins in gastric mucosal biology. Here, we report that RegIII proteins levels are greatly induced in inflamed gastric mucosa, which suggests that they may play a role in stimulating gastric epithelial cell proliferation.

In this paper, we examined a number of factors that may be involved in causing gastric hypertrophy in mice with autoimmune gastritis. Gastrin was examined because, as indicated above, its levels are elevated in autoimmune gastritis, and we had recently shown that gastric mucosal hypertrophy in mice deficient in the gastric H+-K+-ATPase β-subunit was completely dependent on elevated gastrin levels (9). Furthermore, the availability of gastrin-deficient mice allowed us to functionally determine its role in gastritis. We found that the pathological features of autoimmune gastritis in mice deficient for gastrin were identical to that normally found. We also determined whether levels of other growth factors known to induce gastric proliferation were increased. We found that expression of the Reg genes, in particular, were greatly elevated and that this increase was independent of gastrin.

MATERIALS AND METHODS

Animals. Gastrin-deficient mice are as described previously (10) and were back-crossed to BALB/cCrSlc greater than five times before experimentation. All mice were housed at the Department of Biochemistry and Molecular Biology, University of Melbourne animal facility under conventional conditions.

Induction and analysis of autoimmune gastritis. Autoimmune gastritis was induced by thymectomy of mice 3 days after birth under cold anesthesia as described previously (3).

Autoantibodies to porcine H^+\text{-}K^+\text{-}ATPase present in mouse sera were detected using an ELISA as described previously (3). For histological assessment, stomachs from 12- to 17-wk-old mice were washed in PBS, fixed in 10% buffered formalin, and embedded in paraffin wax. Sections (4 μm) were cut, dewaxed, cleared, and stained in hematoxylin and eosin. Cell types of the gastric mucosa were identified by their morphological characteristics as previously described (15). For quantitative purposes, five sections from each stomach at least 30 μm apart were analyzed. From each section, the lengths of four randomly chosen gastric units were measured using Image-Pro software (Media Cybernetics). Only complete oxyntic units were analyzed.

Immunohistochemical detection of mitosis. Stomachs from mice were washed in PBS, fixed in 10% buffered formalin, and embedded in paraffin wax. Dewaxed and cleared sections (4 μm) were incubated in DAKO antigen retrieval solution (DAKO S1700) at 98°C for 25 min. Sections were allowed to cool for 20 min before incubation with an anti-PCNA specific antibody (DAKO M0879; 0.48 mg/ml), followed by a streptavidin-horseradish peroxidase complex (Amersham 140169). Bound horseradish complex was detected by incubation with PBS containing 0.05% dianisobenzidine and 0.03% nickel chloride for 11 min. Sections were then counterstained for 30 s with haematoxylin.

RNA analysis. cDNA fragments of RegI, amphiregulin, HB-EGF, EGF receptor, TGF-α, and the housekeeping gene ribosomal protein L32 (rL32) were generated by RT-PCR using the following primer pairs: RegI (forward) 5’-CATCCTGTCTCCTGCTGATCT-3’ (reverse) 5’-GAAGCAAGTTGTCTCTCCAGG-3’, RegII (forward) 5’-ATTATTGATTTGAAATTTAAA-3’, (reverse) 5’-GAGTTTCTGACATCTGTTC-3’, RegIII (forward) 5’-CATACTCTAGCATCAATAG-3’ (reverse) 5’-AAAATGAGAAGAAGAAGGAA-3’, RegIV (forward) 5’-TCATCTCTACGAGAAGAAGGAA-3’ (reverse) 5’-TTGGGATGCCCATTTCCTTTCC-3’, TGF-α (forward) 5’-GACGCTGCCTGAGAAATG-3’ (reverse) 5’-CTCTGGATCTTCGACAGC-3’, rL32 (forward) 5’-AACCCAGAGCAGTCGACAACA-3’ (reverse) 5’-GAACACAAAAACAGGCACACA-3’. PCR fragments were purified using Qiagen (QIAquick) PCR purification kit (catalog no. 28104). RegIIβ and RegIIIβ probes were generated from I.M.A.G.E. consortium clones purchased from Incyte genomics. Purified cDNA (100 ng) was labeled using random primers and [α-32P]dCTP as previously described (24). Unincorporated α-32P was removed by using NucTrap (Stratagene). RNAs from pancreas and duodenum, tissues that express all Reg family members, were included in blotting experiments.

Total RNA was extracted from the gastric fundus of 12-wk-old unmanipulated and day 3-thymectomised heterozygous and gastrin-deficient mice using TRIzol (Invitrogen Life Technologies) following the manufacturer’s instructions. A total of 20 μg of RNA in glyoxyl sample buffer (BioWhittaker Molecular Applications) was electrophoresed in 1% agarose/20 mM 3-N-Morpholinopropanesulfonic acid gel and transferred to nitrocellulose membrane (Hybond) before crosslinking by ultraviolet irradiation at 150 mJ. Membranes were hybridized for at least 2 h with 32P-labeled cDNA (2 × 106 counts/min·μl) using RapidHyb solution (Amersham Biosciences) as per the manufacturer’s instructions. Blots were washed in 2–0.5% SSC/0.1% SDS at 65°C and exposed to X-ray film at –80°C. To correct for RNA loading and transfer, membranes were stripped and rehybridized with a complementary cDNA probe to rL32. X-ray films were scanned, and band intensities were measured using ImageMaster 1D software (Amersham-Biosciences). Relative expression units were derived by dividing the band intensities obtained from using the individual cDNAs by the band intensities for rL32. In situ hybridization using digoxigenin-labeled cRNAs was used to localize RegIIβ mRNA in mouse stomach sections using procedures previously described (23) with hybridization and washing temperatures at 55°C. cRNAs were made by transcription of mouse RegIIβ PCR products generated from a plasmid kindly provided by Prof. Hiroshi Okamoto (Tohoku University, Miyagi, Japan). Both antisense and sense cRNAs were used on each sample in every experiment for each set of conditions tested. All experiments were performed with the approval of the University of Melbourne Animal Experimentation Ethics Committee.

RESULTS

Gastrin is not required for the development of chronic gastric autoimmunity. Thymectomy of certain strains of mice at day 3 after birth induces autoimmune gastritis with high incidence, and this is a well-defined model for this disease (12, 28, 30). To determine whether the presence or absence of gastrin affected the incidence or course of autoimmune gastritis, we thymectomized cohorts of gastrin-deficient or wild-type mice at day 3 after birth. The mice had been back-crossed to the BALB/cCrSlc mice, a strain with high incidence of gastritis (26), at least five generations before experimentation. At 12 wk of age, serum autoantibodies directed to the major gastric autoantigen, the gastric H^+\text{-}K^+\text{-}ATPase, were detected by
ELISA. In mice of both genotypes, the incidence of gastric autoantibodies was in the range expected for this mouse strain (wild type 65%, gastrin-deficient 76%; Fig. 1A).

Gastric inflammation (gastritis) was analyzed by examining stained sections of tissue. All mice, except one, that had serum autoantibodies also had gastritis (Fig 1B), as determined by the observation of mononuclear cells in the mucosa and alterations in the structure of the gastric mucosal tissue (see Pathological features of autoimmune gastritis do not require gastrin).

These data indicate that autoimmune responses and the incidence of gastritis were not altered by the presence or absence of gastrin. Pathological features of autoimmune gastritis do not require gastrin. We have previously characterized the mucosal lesion of mice with autoimmune gastritis in some detail (15). In that study, we found that normal gastric mucosal cell development was disrupted and the predominant pathological feature was an amplification of immature cell types that resemble undifferentiated gastric mucosal stem cells, which resulted in a pronounced gastric mucosal hypertrophy. In addition, the numbers of parietal and zymogenic cells were severely decreased. In a minority of gastric units, large cells with cytoplasm packed with mucus granules, so called “mucus-rich” cells (15), were the most numerous cell type instead of immature cells. The presence of a mononuclear cell infiltrate is also a hallmark of this disease. Stained sections of the stomach of thymectomized wild-type and gastrin-deficient mice were examined for these features of autoimmune gastritis (Fig. 2). All of these features were also observed here in both the gastritic wild-type and gastritic gastrin-deficient mice. As previously observed, the gastric mucosa of unmanipulated wild-type and gastrin-deficient mice is indistinguishable by light microscopy (Fig. 2, A and B) (9). In mice of both genotypes with autoimmune gastritis, gastric mucosal hypertrophy was observed (Fig 2, C and D), and in most gastric units, this was the result of large numbers of immature gastric epithelial cells (Fig. 2, E and F). Few parietal and zymogenic cells were observed, and there was accompanying mononuclear infiltrate in the submucosa extending into the mucosal lamina propria (Fig. 2, G and H). In a minority of gastric units, mucus-rich cells were prevalent (Fig. 2, G and H). In some mice (~17% in both genotypes), the gastric mucosa was infiltrated with mononuclear cells, but hypertrophy was not observed (Fig. 2, I and J).

To obtain a quantitative measure of the pathological changes in mice with gastritis, we measured the length of the gastric units in stomachs with no evidence of autoimmune gastritis (i.e., no gastric inflammation or autoantibodies) and stomachs with hypertrophic gastritis derived from mice of both genotypes (Fig. 3). Sections from the stomachs of three mice per group were analyzed as described in MATERIALS AND METHODS. The lengths of the gastric mucosae of the wild-type nongastritic and gastrin-deficient nongastritic mice were very similar. The lengths of the mucosae of the wild-type gastritic and gastrin-deficient gastritic mice were also very similar, and they were 1.6-fold longer than nongastritic mice (P < 0.01, ANOVA on ranks).

The titers of the anti-H⁺-K⁺-ATPase autoantibodies in mice with autoimmune gastritis of both genotypes were also determined as another quantitative measure of disease (Fig. 4). There was no significant difference between titers in the two genotypes (P = 0.71; Mann-Whitney rank sum test).

To detect proliferating cells, sections of gastric mucosa were stained with an antibody directed to the mitosis-associated antigen PCNA (Fig. 5). As expected from previous work, the majority of dividing cells in the gastric mucosa of wild-type and gastrin-deficient nongastritic mice were in the isthmal region of the mucosa, which is the location of the majority of the proliferative immature cells (Fig. 5, A and B) (9, 15, 18). In the mucosa of the wild-type gastritic and gastrin-deficient gastritic mice, PCNA-staining cells were much more numerous and were located in the one-third to one-half of the units closest to the lumen that were rich in the amplified immature cell population (Fig. 5, C and D). These data suggest that in both genotypes, the hypertrophy was due primarily to greater rates of mucosal cell proliferation and that this proliferation was induced by factors other than gastrin.

Hence, the pathological features of autoimmune gastritis are not dependent on the presence of gastrin. Analysis of expression of factors potentially involved in regulating gastric epithelial cell growth. To search for other molecules that may induce proliferation of gastric mucosal...
cells in mice with autoimmune gastritis, we measured expression of gastric growth factors by RNA hybridization experiments. Gastrin-deficient mice were thymectomized on day 3 after birth, and stomachs were removed for histological analysis at 12 wk of age. Tissues were fixed, sectioned, and stained with hematoxylin and eosin and examined by light microscopy. The lumen is at the top of each panel. Gastric mucosae of unthymectomized wild-type (A) and gastrin-deficient (B) mice appeared to have identical structure. Gastric mucosae of wild-type gastritic (C) and gastrin-deficient gastritic mice (D) were usually hypertrophic. Immature cells, as identified by a large nuclear-to-cytoplasmic ratio and a cytoplasm that stained strongly with eosin, predominated in gastric units from wild-type gastritic (E) and gastrin-deficient gastritic mice (F) mice. In some mice, gastric units are “mucus-rich” cells seen in wild-type gastritic (G) and gastrin-deficient gastritic mice (H). A few wild-type mice developed gastric mucosal mononuclear cell infiltrate (I and J, arrows indicate infiltrate) but not hypertrophy (gastritic/nonhypertrophic). Bar in A = 50 μm and applies to A-D and I and J. Bar in E = 10 μm and applies to E-H.
We observed no significant variation in the levels of mRNAs encoding RegII, RegIIIα, RegIV, TGF-α, HB-EGF, or the EGF receptor for any of the samples (data not shown). The levels of RegII, RegIIIα, RegIV mRNAs were very low in all samples.

Representative autoradiograms of the RNA blots and quantitation of hybridization that displayed significant changes between the groups are shown in Fig. 6. Consistent and very large increases were observed in the RNA samples derived from the hypertrophic stomachs of both the gastrin-deficient mice and their heterozygous littermates for the mRNAs encoding RegIIIα (average values increased 31- and 45-fold for gastrin heterozygotes and gastrin-deficient mice, respectively) and RegIIIγ (average values increased 49- and 62-fold for gastrin heterozygotes and gastrin-deficient mice, respectively). RegI levels were also increased, although not to the same extent (average values increased 16- and 4.2-fold for gastrin heterozygotes and gastrin-deficient mice, respectively).
Amphiregulin mRNA levels were elevated in two of the gastrin heterozygote gastritic/hypertrophic mice but not the third. Amphiregulin mRNA levels were greater in the gastrin-deficient gastric/hypertrophic mice than in the gastrin heterozygote gastric/hypertrophic mice.

The levels of mRNAs encoding the factors were very similar in the nongastritic mice and in the mice with gastric mononuclear cell infiltrate but no hypertrophy (gastrin heterozygote gastric/hyperplastic). Hence, it appears that elevated levels of Reg and amphiregulin are associated with hypertrophy and not gastric inflammation per se.

Analysis of cells expressing RegIIIβ in inflamed gastric mucosa. To discover which cells in the gastric mucosa of gastritic mice express RegIIIβ, we hybridized tissue sections with cRNAs corresponding to the sequence of RegIIIβ (Fig. 7).

Hybridization with sense cRNA resulted in negligible staining (Fig. 7A). Antisense cRNA hybridized to a large number of epithelial cells (Fig. 7B). The cells expressing the RegIIIβ mRNA appeared to be small immature cells, as judged by their morphology and position in the gastric units (Fig. 7C). The location of cells binding the RegIIIβ cRNA was similar to the location of cells that stained with PCNA (Fig. 5), supporting their assignment as immature cells. Another prominent cell population in the units of mice with autoimmune gastritis is mucus-rich cells (15). These cells did not appear to express RegIIIβ mRNA (Fig. 7D).

DISCUSSION

Gastric epithelial hypertrophy is one of the dominant features of autoimmune gastritis and is the result of the accumulation of large numbers of immature epithelial cells (15). A number of factors has been implicated in stimulating cell growth in the gastric mucosa including gastrin, RegI, and EGF receptor ligands. In this study, we have sought to discover which of these molecules are involved in inducing the gastric mucosal hypertrophy in autoimmune gastritis.

Previous investigations (21) had indicated that gastrin levels were elevated in mice with autoimmune gastritis. Hence, we examined the contribution of this hormone to the pathological features of autoimmune gastritis by using gastrin-deficient mice. When gastrin-deficient mice were thymectomized on day 3, we found that both the incidence of mononuclear cell infiltrate and anti-H⁺-K⁺-ATPase autoantibodies, two hallmarks of autoimmune gastritis, were very similar to those of wild-type mice of a similar genetic background (Fig. 1). Hence, gastrin is not required for the development of an autoimmune response to gastric autoantigens and development of chronic disease.

Gastrin was also not required for the pathological features observed in mice with autoimmune gastritis. Gastrin-deficient mice had the same degree of gastric mucosal hypertrophy, as measured by an increase in gastric mucosal unit length, as wild-type animals (Fig. 3). Anti-H⁺-K⁺-ATPase antibody levels were also quantitated (Fig. 4), and no differences were found between mice of the two genotypes. Gastric mucosal hypertrophy in wild-type mice with autoimmune gastritis is primarily the result of the accumulation of abnormally high levels of immature epithelial cells (15) that accumulate due to increased levels of cellular proliferation. The gastric mucosa of gastrin-deficient gastritic mice also had increased numbers of small immature cells (Fig. 2), and an elevated number of cells in mitosis was detected (Fig. 5). In a minority of gastric units from mice with autoimmune gastritis, large mucus-rich cells accumulate (15) that resemble the recently described spasmyotic polypeptide-expressing metaplasia lineage (32). These cells also appeared in gastrin-deficient mice (Fig. 2). Finally, gastrin-deficient mice also displayed depletion of parietal and zymogenic cells, another feature of autoimmune gastritis (Fig. 2). From these analyses, we conclude that gastrin does not contribute to the pathological features of autoimmune gastritis. This contrasts with our recent data (9) in another system of gastric mucosal hypertrophy, the H⁺-K⁺-ATPase β-subunit-deficient mouse, in which the severe hypertrophy was entirely gastrin dependent. Clearly, various pathways in-
volving different mitogenic stimuli may lead to disruption of gastric epithelial cell homeostasis.

Ligands of the EGF receptor have been implicated in inducing gastric cell proliferation and function. We found that the EGF receptor ligands TGF-α and HB-EGF mRNA levels were not significantly elevated in mice with autoimmune gastritis. Hence, we believe it is unlikely that these molecules are responsible for the observed hypertrophy. Levels of another EGF receptor ligand amphiregulin were increased by 3.7- and 2.5-fold in two of three gastrin-heterozygote hypertrophic mice but were not elevated in one mouse. This relatively small and variable increase suggests that this hormone may play only a minor role in hypertrophy observed in this system. The increase in amphiregulin levels in gastrin-deficient gastritic/hypertrophic mice was greater than in the gastri-hypertrophic mice but were not elevated in one mouse. This increase in amphiregulin levels in gastrin-deficient gastritic/hypertrophic mice was greater than in the gastrin-deficient gastritic/hypertrophic mice (average values increased 2.1-fold in gastrin heterozygotes and 5.5-fold in gastrin-deficient mice). This suggests that gastrin may negatively regulate release of this hormone in inflammatory situations or that amphiregulin levels are increased to compensate for a lack of gastrin. It has been suggested that parietal cells are primarily responsible for the production of amphiregulin (2). Because parietal cells are severely depleted from mice with severe autoimmune gastritis, it would appear likely that other cell types are also responsible for its production in this inflammatory situation.

RegI was initially described as an inducer of pancreatic islet cell proliferation. Subsequently, several members of the Reg family have been identified in humans, rats, and mice with 40–70% identity between family members (1, 25). Analysis of the mouse genome has identified four Reg subfamilies (1, 13). RegI (pancreatic stone protein) and RegII are the only members of their subfamilies. The RegIII subfamily has four members: RegIIIα, RegIIIβ, RegIIIγ, and RegIIIδ. RegIIIα, RegIIIβ, and RegIIIγ appear to have similar tissue distributions and are expressed at significant levels in the gastrointestinal tract. RegIIIδ, on the other hand, appears to be expressed only in the pancreas (1). RegIV is also expressed throughout the gastrointestinal tract (13). Recently, a Reg-like protein with ~45% identity to Reg proteins has also been identified, which is also expressed in the gastrointestinal mucosa (17).

To date, little emphasis has been placed on examining the role of members of the Reg family apart from RegI in the gastric mucosa. We examined expression of RegI, RegII, RegIIIα, RegIIIβ, RegIIIγ, and mRNA. RegII, RegIIIα, and RegIV mRNAs were very low to undetectable in stomachs from all mouse groups. In contrast to this were the levels of RegIIIβ and RegIIIγ mRNAs, which were increased 30- to 60-fold in all of the gastritic/hypertrophic mice. RegI mRNA levels were more modestly increased (4- to 16-fold). Importantly, we also examined mice that had a mononuclear cell infiltrate but had not developed hypertrophy. In these mice, the level of the Reg mRNAs were not significantly different from mice without gastritis, indicating a strong correlation with hypertrophy and increased Reg levels rather than just with the presence of a mononuclear infiltrate. These data were the first demonstration of the induction of RegIIIβ and RegIIIγ mRNAs in inflamed gastric mucosa, and they suggest that these genes may play a significant role in the pathological manifestations of autoimmune gastritis, whereas other Reg family members, namely RegII, RegIIIα, and RegIV are unlikely to be involved. It is also noteworthy that the genes for the RegIIIs can be regulated independently despite their close proximity.

Fig. 7. In situ hybridisation with RegIIIβ cRNA. Wild-type mice were thymectomized at day 3 after birth, and stomachs were removed for analysis at 12 wk of age. Tissues were fixed, sectioned, and incubated with labeled cRNA probes corresponding to the sense (A) or antisense (B–D) sequences of RegIIIβ. C and D are higher magnification images of the boxed areas indicated in B. Bar in A = 50 μm and applies to A and B. Bar in D = 10 μm and applies to C and D.
In situ hybridization with a RegIIIβ cRNA demonstrated that an abundant population of epithelial cells in the gastritic mucosa expressed the RegIII mRNA. These cells were small and were positioned immediately below the pit cell region in the area containing cells that stained with the mitosis associated-antigen PCNA. Thus these cells are likely to be immature cells. Immature cells are the most abundant cell type in the mucosa of gastritic mice; hence, elevated expression in this cell population is consistent with the abundance of the RegIII transcripts. Expression of the RegIII genes in the rapidly proliferating immature cells of the mucosa raises the possibility that proliferation is driven by an autocrine loop.

This work has demonstrated that gastrin is not involved in the pathological features of autoimmune gastritids and that members of the Reg family are strong candidates to stimulate the proliferation of immature cells. Importantly, this study has shown that gastrin is not essential for the production of Reg proteins. Recently, it was demonstrated that cytokines associated with inflammation, such as CINC-2β (19), TGF-α, and interferon-γ, are able to induce production of Reg I and RegIII (pancreatitis-associated protein I) (8) in rats. Previously, it was shown that the proliferation of immature cells, such as CINC-2β induces the expression of RegIII genes in the rapidly proliferating immature cells of the mucosa raises the possibility that proliferation is driven by an autocrine loop.

REFERENCES


