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Anti-Inflammatory Activity of Bone Morphogenetic Protein Signaling Pathways in Stomachs of Mice

Hidehiko Takabayashi[1,*], **Masahiko Shinohara**[1,*], **Maria Mao**[1], **Piangwarin Phaosawasdi**[1], **Mohamad El-Zaatari**[1], **Min Zhang**[1], **Tuo Ji**[1], **Kathryn A. Eaton**[2], **Duyen Dang**[1], **John Kao**[1], and **Andrea Todisco**[1]

[1]Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, Michigan

[2]Laboratory Animal Medicine Unit, University of Michigan Medical Center, Ann Arbor, Michigan

Abstract

BACKGROUND & AIMS—Bone morphogenetic protein (BMP)4 is a mesenchymal peptide that regulates cells of the gastric epithelium. We investigated whether BMP signaling pathways affect gastric inflammation after bacterial infection of mice.

METHODS—We studied transgenic mice that express either the BMP inhibitor noggin or the β-galactosidase gene under the control of a BMP-responsive element and BMP4$^{\beta gal/+}$ mice. Gastric inflammation was induced by infection of mice with either *Helicobacter pylori* or *Helicobacter felis*. Eight to 12 weeks after inoculation, gastric tissue samples were collected and immunohistochemical, quantitative, reverse-transcription polymerase chain reaction and immunoblot analyses were performed. We used enzyme-linked immunosorbent assays to measure cytokine levels in supernatants from cultures of mouse splenocytes and dendritic cells, as well as from human gastric epithelial cells (AGS cell line). We also measured the effects of BMP-2, BMP-4, BMP-7, and the BMP inhibitor LDN-193189 on the expression of interleukin (IL)8 messenger RNA by AGS cells and primary cultures of canine parietal and mucus cells. The effect of BMP-4 on NFkB activation in parietal and AGS cells was examined by immunoblot and luciferase assays.

RESULTS—Transgenic expression of noggin in mice increased *H pylori*– or *H felis*–induced inflammation and epithelial cell proliferation, accelerated the development of dysplasia, and increased expression of the signal transducer and activator of transcription 3 and activation-induced cytidine deaminase. BMP-4 was expressed in mesenchymal cells that expressed α-smooth muscle actin and activated BMP signaling pathways in the gastric epithelium. Neither BMP-4 expression nor BMP signaling were detected in immune cells of C57BL/6, BRE–β-galactosidase,

Reprint requests Address requests for reprints to: Andrea Todisco, MD, 6520 Medical, Science Research Building I, Ann Arbor, Michigan 48109-0682. atodisco@umich.edu; fax: (734) 763-2535.
*Authors share co-first authorship.

Supplementary Material
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Conflicts of interest
The authors disclose no conflicts.

or BMP-4$^{\beta gal/+}$ mice. Incubation of dendritic cells or splenocytes with BMP-4 did not affect lipopolysaccharide-stimulated production of cytokines. BMP-4, BMP-2, and BMP-7 inhibited basal and tumor necrosis factor α–stimulated expression of IL8 in canine gastric epithelial cells. LDN-193189 prevented BMP4-mediated inhibition of basal and tumor necrosis factor α–stimulated expression of IL8 in AGS cells. BMP-4 had no effect on TNFα-stimulated phosphorylation and degradation of IκBα, or on TNFα induction of a NFκβ reporter gene.

CONCLUSIONS—BMP signaling reduces inflammation and inhibits dysplastic changes in the gastric mucosa after infection of mice with *H pylori* or *H felis*.

Keywords

BRE; STAT3; Differentiation; Immune Regulation

The bone morphogenetic proteins (BMPs) are important regulators of a broad array of biological actions during both embryonic and postnatal vertebrate development.[1–13] BMP-2, BMP-4, and BMP-7 appear to be significantly expressed in gastrointestinal tissues, where they have been shown to play a significant role in the regulation of cellular proliferation and differentiation.[1–13] The clinical relevance of these observations has been underscored by studies conducted in human tissues that have demonstrated an important role for BMP signaling in the inhibition of gastrointestinal tumor growth.[4,9–12]

The actions of the BMPs can be specifically blocked, by inhibitory proteins that are expressed in tissues to modulate the level of activation of BMP signaling.[1–14] Of these, noggin, a secreted polypeptide present in several mammalian tissues, has been shown to bind to, and inhibit, the actions of BMP-2, BMP-4, and, to a lesser degree, BMP-7.[1,2,5,14] In addition, small molecule inhibitors of BMP type I receptors have been recently described.[15] These compounds have been employed both in vivo and in vitro to manipulate a broad array of physiological functions, underscoring the importance of BMP signaling in both physiology and disease.[15]

BMP-4, in particular, appears to be expressed in the mesenchymal layers of the gastric mucosa and to exert significant regulatory effects on gastric physiology.[3,16–18] Studies from our laboratory have shown that incubation of cultured parietal cells with this peptide leads to stimulation of H^+/K^+-adenosine triphosphatase α-subunit gene expression and to enhancement of secretagogue-stimulated gastric acid production.[16] In a series of recent in vivo investigations, we and others also have shown that inhibition of BMP signaling in the gastric epithelium causes profound aberrations in the normal mechanisms that regulate the proliferation, maturation, and differentiation of several lineages of gastric epithelial cells, underscoring the importance of BMP signaling in the regulation of gastric epithelial homeostasis.[17,18]

In addition to these effects, studies have shown that the BMPs might exert widespread anti-inflammatory actions and therefore they could represent novel and hitherto poorly characterized regulators of gastrointestinal inflammation.[19–21] In support of this hypothesis, the gastric mucosa of patients infected with *Helicobacter pylori* shows increased expression of both BMP-2 and BMP-4,[22] indicating that these peptides might be involved in

Helicobacter-induced gastric inflammation. Similarly, BMP-7 has been shown to ameliorate the severity of colonic inflammation and to accelerate the healing of colitis in rats exposed to trinitrobenzene sulfonic acid, a well-established inducer of experimental colitis in rodents.[19,20] Finally, transgenic expression of BMP-4 in the skin of mice treated with both the carcinogen N-methyl-N'-nitrosoguanidine and the tumor promoter 12-O-teradecanoylphorbol-13-acetate leads to a marked decrease in the degree of cellular hyperproliferation and inflammation induced by these agents.[24]

Exposure of the gastric epithelium to inflammatory stimuli leads to the release of cytokines and chemokines, peptides known to play a prominent role in the induction and maintenance of gastric mucosal damage.[23–27] The chemokine Interleukin (IL)8, in particular, is one of the best-characterized mediators of *Helicobacter*-induced gastric inflammation.[23–26] IL8 is released by cells of the gastric epithelium in response to both infection with *Helicobacter* and to stimulation with IL1β, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ),[23–27] cytokines released by activated macrophages and T helper 1 (Th1) lymphocytes, respectively.

Although the significance of gastric inflammation in the pathogenesis of peptic ulcer and gastric cancer has been appreciated, the factors and the signaling pathways involved in the development of these diseases only partially have been characterized. In particular, the function and localization of BMP-4 and the cellular targets of the BMP signal transduction pathway in the inflamed stomach currently are unknown.

Accordingly, we took advantage of several lines of genetically engineered mice and of well-established primary cultures of gastric epithelial cells to test the hypothesis that BMP-4 expression and signaling are modulated by inflammation and that the BMP signal transduction pathway negatively regulates the response of the gastric mucosa to inflammatory stimuli.

Material and Methods

Mice

See Supplementary Materials and Methods.[17,28,29]

H pylori and *Helicobacter felis* Culture and Infection

See Supplementary Materials and Methods.[30,31]

H pylori Lipopolysaccharide Isolation

See Supplementary Materials and Methods.[30,31]

Primary Cell Culture

See Supplementary Materials and Methods.[16]

Generation of Bone Marrow–Derived Dendritic Cells

See Supplementary Materials and Methods.[32,33]

Quantitative Reverse-Transcription Polymerase Chain Reaction Analysis

See Supplementary Materials and Methods.[16,17]

Enzyme-Linked Immunosorbent Assay

See Supplementary Materials and Methods.

Histochemical Analysis and Image Acquisition

See Supplementary Materials and Methods.[17,28,33,34]

Northern Blots

See Supplementary Materials and Methods.[16]

Western Blots

See Supplementary Materials and Methods.[16,17]

Data Analysis

Data are expressed as means ± standard error. Statistical analysis was performed using the Student *t* test. *P* values less than .05 were considered significant.

Results

In order to test the hypothesis that the BMPs inhibit gastric inflammation, we took advantage of the promoter of the mouse H^+/K^+-ATPase β-subunit gene to express the secreted BMP inhibitor noggin in the gastric epithelium of mice.[17] Microscopic analysis of H&E-stained sections of the fundic mucosa of the transgenic, but not of wild-type, control mice (Figure 1A) revealed the presence of foci of mild to moderate inflammatory infiltrates (Figure 1B–D). Measurement by QRT-PCR of TNF-α, IFN-γ, macrophage inflammatory protein-2 (MIP-2), and IL1β messenger RNAs (mRNAs) demonstrated that inhibition of BMP signaling causes a significant increase in the expression of these inflammatory molecules (Figure 1E). In contrast to these findings, a previously published study indicated that transgenic expression of noggin in the gastric epithelium by means of the Keratin 19 promoter (K19-Nog mice) does not lead to the expression of a significant gastric phenotype.[35] As previously reported,[17] it is possible that this discrepant phenotypic outcome might have been due to differences between our transgenic vector and that used in the K19-Nog mice.

To better characterize the significance of these observations we challenged 3-month-old noggin transgenic mice with the Sydney strain 1 (SS1) strain of *H pylori*, which is known to induce an inflammatory response in the gastric mucosa of mice.[30–34] Microscopic and morphometric analysis of H&E-stained sections of the fundic mucosa of the transgenic mice 3 months after *H pylori* infection showed a significant increase in the severity of the inflammatory infiltrates and the presence of areas of dysplastic mucosa when compared with nontransgenic/noninfected, nontransgenic/*H pylori*–infected, and transgenic/noninfected, age-matched littermates (Figure 2A–E).

In agreement with these observations, exposure of the gastric mucosa of the transgenic mice to *H pylori* led to enhanced expression of MIP-2, TNF-α, IFN-γ, and IL1β mRNAs (Figure 3A–D). Thus, inhibition of BMP signaling in the gastric epithelium leads to a proinflammatory state, resulting in extreme responses and in accelerated development of dysplasia with *H pylori* infection.

In order to investigate if enhanced inflammation leads to increased cell proliferation, we stained sections of the fundic mucosa with antibodies directed against both proliferating cell nuclear antigen and Ki 67[17] (and data not shown), well-established markers of cell proliferation. As shown in Figure 4A and data not shown, in agreement with previously published reports, both infection with *H pylori*[33,36] and inhibition of BMP signaling[17] led to a significant increase in the number of proliferating cell nuclear antigen–positive nuclei, an effect that was enhanced markedly by infection of the transgenic mice with *H pylori* (Figure 4A–C). We then examined the role of BMP signaling on the expression of molecules, such as STAT3, which are known to mediate inflammatory and proliferative signals in the gastric mucosa.[37] Accordingly, using Western blots with anti–phospho-STAT3 antibodies, we measured the activation of STAT3 in the gastric mucosa of both transgenic and nontransgenic mice in the presence and absence of *H pylori*. As depicted in Figure 4D, infection of the transgenic mice with *H pylori* led to a dramatic increase in the level of phosphorylation of STAT3. In agreement with these observations, immuno-histochemical analysis with anti-P-STAT3 antibodies, confirmed the presence of positively stained nuclei in clusters of inflammatory and epithelial cells in the *H pylori*-infected transgenic-mice but not in the other groups of animals (Supplementary Figure 1A and B and data not shown). Similar results were observed when we measured by QRT-PCR the expression of AID, a molecule that has been shown to mediate some of the pro-oncogenic actions of *H pylori* in the stomach[38] (Figure 4E). Thus, inhibition of BMP signaling and heightened gastric inflammation induce the development of a pro-oncogenic environment characterized by increased cell proliferation and by enhanced expression of STAT3 and AID.

Trefoil factor 2 (TFF2) is a peptide growth factor that, in the normal stomach, is expressed in deep gland cells of the antrum and in the mucus neck cells of the fundic glands.[17,39] Moreover, aberrant expression of TFF2 at the base of the fundic glands has been associated with the occurrence of spasmolytic polypeptide-expressing metaplasia, an event linked to the development of both dysplasia and neoplasia in *Helicobacter*-infected mice.[39,40] In previously published studies we reported that inhibition of BMP signaling in the stomach leads to increased TTF2 mRNA expression and spasmolytic polypeptide-expressing metaplasia.[17] In this report we observed that *H pylori* infection significantly enhanced the expression of TFF2 mRNA in transgenic mice when compared with both infected nontransgenic, and noninfected transgenic controls (Supplementary Figure 2A). In contrast, *Helicobacter* infection did not alter the level of MUC6 mRNA, another mucus neck cell marker whose expression is increased significantly in transgenic mice (Supplementary Figure 2B). Thus, inhibition of BMP signaling and *H pylori*–induced inflammation appear to exert a synergistic and specific effect on TFF2 mRNA expression.

We examined the localization of BMP-4–expressing cells in the gastric mucosa both in the presence and absence of inflammation. As shown in Figure 5A, X-gal staining of the gastric

mucosa of BMP-4$^{\beta gal/+}$ mice indicated that BMP-4 is expressed in mesenchymal cells located both under and between the glands. To analyze the pattern of expression of BMP-4 during inflammation, we infected the BMP-4$^{\beta\text{-}gal/+}$ mice with *H felis* for 2 months. For these studies we elected to use *H felis* because it induces more rapid and robust inflammation than *H pylori* in mice.[36] *H felis* induced the expression of TNF-α, MIP-2, and IFN-γ mRNAs in these mice (data not shown), and, as shown on the representative H&E-stained frozen sections depicted in Figure 5B, it led to the development of significant foci of inflammatory infiltrates in the mucosa of the corpus. Immunohistochemical analysis of these sections using anti-F4/80 antibodies confirmed the presence of inflammatory cells (Supplementary Figure 3). Moreover, staining of corresponding sections with X-gal showed, as depicted in Figure 5C, that BMP-4 is expressed in clusters of cells that appear to be localized in the mesenchymal layers of the mucosa, adjacent to, but not in the inflammatory infiltrates. To define the localization of cells receiving BMP-generated signals, we used BRE–β-galactosidase mice. As shown in Figure 5D, X-gal positively stained cells could be detected, mostly at the level of the isthmus and neck of the glands, but not in the mesenchyme, both in the absence (Figure 5D) and presence of inflammation, as shown in the H&E- and matching X-gal–stained sections shown in Figure 5E and F. Staining with anti-H^+,K^+-ATPase a-subunit antibodies confirmed that these sections were obtained from the oxyntic mucosa (Supplementary Figure 4). Similar results were observed when we stained paraffin sections of the gastric mucosa of *H pylori*–infected mice with antibodies recognizing phosphorylated and active forms of the BMP-4 signal transducing proteins, Smad1, 5, and 8[1,2] (Supplementary Figure 5A and B). Thus, studies conducted with 2 different experimental approaches, in the presence of 2 types of *Helicobacter* organisms, confirmed the notion that BMP-generated signals specifically target cells located in the epithelium but not in the mesenchyme or in the inflammatory infiltrates.

To better define the type of cells that express BMP-4 in the inflamed stomach, we performed an immunohistochemical analysis of sections of the fundic mucosa of *H felis*–infected BMP-4$^{\beta\text{-}gal/+}$ mice. Although, as indicated in Figure 6A, BMP-4 expression could be detected predominantly in α-smooth muscle antibody (SMA)–positive cells, a few rare β-galactosidase–positive, α-SMA–negative cells also were noted, suggesting that BMP-4 expression is not restricted to α-SMA–expressing mesenchymal cells. Similar results were observed in the gastric mucosa of noninfected animals (data not shown). No significant BMP-4 expression could be identified in cells expressing macrophage, B, T, dendritic, and neutrophil markers (Figure 6B–F). Thus, myofibroblasts, but not immune cells, appear to represent the main source of BMP-4 expression in the gastric mucosa.

Helicobacter-induced inflammation is a complex process that involves the activation of several types of inflammatory cells.[36] Among these, dendritic cells are known to play a crucial role in the pathophysiology of the gastric inflammatory response.[30–32,36] To confirm that BMP-4–generated signals do not target inflammatory cells, we treated cultures of dendritic cells with *H pylori* LPS, either alone or in association with BMP-4. As shown in Supplementary Figure 6A and B, BMP-4 did not have any effect on both basal and LPS-stimulated IL12 and IL23. We investigated whether BMP-4 could modulate the process of immune cell stimulation by activated dendritic cells. Accordingly, we cultured populations

of freshly isolated splenocytes, which are enriched in CD4+ T lymphocytes, together with dendritic cells that were primed with *H pylori* LPS. As shown in Supplementary Figure 6C and D, BMP-4 had no effect on IFN-γ and IL17-α release from dendritic cell–activated splenocytes, confirming the notion that BMP-4 does not modulate the function of these immune cells. Similar results were observed in experiments performed using flow cytometry, in which BMP-4 did not have any significant effect on the percentage of CD4+ T lymphocytes that were primed to produce IFN-γ in response to LPS stimulation (data not shown).

We investigated if the BMPs exert direct inhibitory effects on the expression of proinflammatory molecules in gastric epithelial cells. Accordingly, we performed experiments using primary cultures of canine parietal and mucus cells. We elected to use these cell types because we showed that these cells appear to be the major target of BMP-generated signals in the gastric mucosa of mice.[17] We showed that BMP-4, BMP-2, and BMP-7 are all capable of stimulating Smad1, 5, and 8 phosphorylation after 24, 48, and 72 hours, respectively, in the parietal cells[16] (and data not shown). Similar results were observed in mucus cells stimulated with these BMPs for 24, 48, and 72 hours (data not shown). Moreover, we observed that the stimulatory action of BMP-4 on P-Smad1, phosphorylation was completely blocked by treatment of the cells with noggin (Supplementary Figure 7A). The specificity of this effect was underscored by the observation that noggin failed to block transforming growth factor-β–induced phosphorylation of Smad2 (Supplementary Figure 7B). We examined if the BMPs inhibit the expression of the IL8 gene, a well-established mediator of *Helicobacter-induced* gastric inflammation.[23–27,36] As shown on Northern blots and in the QRT-PCR assays depicted in Figure 7A and B, BMP-2, BMP-4, and BMP-7 significantly inhibited both basal and TNF-α–stimulated IL8 gene expression after 72 hours of incubation in both cell types. Exposure of the cells to a combination of all 3 BMPs did not induce any potentiating or additive effect, suggesting that these agents are likely to share common signaling mechanisms.

To confirm the significance of this effect in human pathophysiology, we performed experiments with AGS cells, a well-established in vitro system that has been used extensively to study the effects of inflammatory stimuli on the production of chemokines in human gastric epithelial cells.[36] As indicated in Supplementary Figure 8, BMP-4 significantly inhibited both basal and TNF-α–stimulated IL8 gene expression in AGS cells. In addition, LDN-193189, a specific BMP-receptor type 1 inhibitor, blocked BMP-4 inhibition of IL8 gene expression, confirming the specificity of this effect. BMP-4 also interfered with the release of the IL8 protein from the AGS cells (Supplementary Figure 9), underscoring the notion that activation of BMP signaling restrains both the expression and the release of inflammatory chemokines in human epithelial cells.

Discussion

In this article we report a series of novel observations that underscore the importance of BMP signaling in the regulation of gastric inflammation and epithelial homeostasis. In particular, we present evidence, using both in vivo and in vitro approaches, that inhibition of

BMP signaling leads to an enhanced response to inflammatory stimuli and to the accelerated development of dysplastic changes of the gastric mucosa.

Several investigations have underscored the importance of inflammation in the pathophysiology of diseases of the stomach such as gastritis, peptic ulcer, and gastric cancer.[41] One of the best-characterized models of gastric inflammation is that caused by infection of the gastric mucosa with *Helicobacter* organisms such as *H pylori* and *H felis*. Inflammation induced by these organisms can induce profound alterations in the normal homeostatic mechanisms of the gastric mucosa, leading to the development of dysplasia and neoplasia.[40,41] In our study we reported that transgenic expression of the BMP-inhibitor noggin during *H pylori* infection leads to a dramatic increase in the expression of the cytokines IL1β, TNF-α, and IFN-γ, and of the chemokine MIP-2, molecules known to cause significant alterations in epithelial cell differentiation, maturation, proliferation, and apoptosis.[23–27,36] We also showed that inhibition of BMP signaling in the presence of *Helicobacter* organisms leads to the accelerated development of dysplasia, to enhancement of gastric epithelial cell proliferation, and to the expression STAT3 and AID, molecules that are activated during inflammation and that contribute to the development of dysplastic and neoplastic changes.[37,38] STAT3, in particular, is a transcription factor that regulates several growth-regulatory and anti-apoptotic genes such as cyclin D1, c-Myc, survivin, and Bcl-X_L. Activation of STAT3 has been linked to aberrant cellular growth and proliferation and to the development of gastrointestinal neoplasias.[37] Interestingly, STAT3 also has been shown to regulate cellular fate and lineage determination because, in the pancreas, it appears to promote conversion of quiescent adult pancreatic epithelial cells to metaplastic cells with a progenitor-like phenotype that is more susceptible to K-Ras–mediated transformation.[37] Similarly, the enzyme AID has been shown to alter P53 function causing significant aberrations in the normal mechanisms that regulate gastric epithelial cell apoptosis during *Helicobacter*-mediated inflammation.[38] Taken together, these observations suggest that BMP signaling inhibits the expression of proinflammatory and pro-oncogenic molecules in the gastric mucosa and that loss of this important homeostatic mechanism might be responsible for the development of metaplastic and dysplastic changes. Another interesting finding of our study was the observation that infection with *Helicobacter* specifically and potently enhances noggin-induced stimulation of TFF2 gene expression. A recent study has suggested that expression of TFF2 mRNA might mark a novel population of gastric progenitor cells that can give rise to several lineages of specialized epithelial cells.[42] An intriguing possibility is that inflammation and lack of BMP signals might be responsible for the expansion of TFF2-labeled progenitors that could, over time, contribute to the development of metaplasia and dysplasia. It is clear that additional experiments with lineage tracing studies will be necessary to test this possibility.

In our article we showed that BMP-4 is a mesenchymal peptide expressed in α-SMA–positive cells and that during inflammation, BMP-4–positive myofibroblasts can be seen in clusters located adjacent to, but not in, the inflammatory infiltrates. Interestingly, we also noted that BMP-4–positive staining could be detected in a few α-SMA–negative cells. Although the meaning of this observation is currently unclear, it is conceivable that BMP-4 could be expressed in different types of mesenchymal cells. Because fibroblasts appear to

play an important role in the generation of cellular niches that can lead to the development of neoplastic transformation,[43] future studies will be directed toward a more in-depth elucidation of the phenotypic and functional characteristics of these cells.

A novel finding of our study was that BMP-4 cannot be detected in cells labeled with macrophage, neutrophil, or T- and B-cell–specific markers, suggesting that BMP-4 is not expressed in these types of inflammatory cells. Interestingly, a previous immunohistochemical study performed in biopsy specimens from patients with *H pylori* gastritis indicated that BMP-2– and, to a lesser degree, BMP-4–positive staining could be seen in cells of the inflammatory infiltrates.[22] Several interpretations could be considered to explain these observations. One possibility is that there could be species-specific differences regarding the expression of BMPs in inflammatory cells. It also is conceivable that BMP-2 and BMP-4 might be expressed in inflammatory cells that do not express F4/80, CD11c, myeloperoxidase, CD19, and CD3. Finally, because the biopsy specimens were not stained with specific immune cell markers, the BMP-positive cells could have represented fibroblasts located in the proximity of the inflammatory infiltrates.

One important finding of our study was that BMP signaling specifically targets epithelial but not mesenchymal and immune cells and that induction of BMP signaling in the epithelium leads to inhibition of IL8 production, a chemokine that plays a crucial role in the response of the epithelium to inflammatory stimuli.[23–27,36] The observation that this effect could be seen in both canine primary epithelial cells and in human AGS cells underscores the relevance of these results in gastric pathophysiology. Accordingly, BMP-4 is likely to represent an anti-inflammatory peptide released by the mesenchyme to modulate the inflammatory response of the gastric epithelium.

The NF-κB signal transduction pathway plays a crucial role in the mediation of TNF-α stimulation of IL-8 gene expression.[26,36] Interestingly, in our study, we observed that BMP-4 did not interfere with the process of IκB-regulated NF-κB activation (see supplemental material). Since induction NF-κB is a complex biological process, [26,36] it is possible that BMP-4 could regulate other mechanisms that lead to the activation of NF-κB. It also conceivable that BMP-4 might cause complex epigenetic modifications of the IL-8 gene that could lead to its inhibition through NF-κB-independent pathways. Elucidation of the mechanisms responsible for BMP-mediated inhibition of IL-8 gene expression will be the focus of future investigations.

An attractive hypothesis is that inflammatory stimuli could induce the expression of BMP-4 and, possibly, of other BMPs, to activate self-safe mechanisms aimed at tampering and limiting the severity of the inflammatory response. The finding that clusters of BMP-4–positive cells can be detected adjacent to the inflammatory infiltrates and the recently published observation that mice infected with *Helicobacter* show increased expression of BMP-4 in the inflamed gastric mucosa, would support this possibility.[44] Another consideration that stems from the finding that there is no BMP-4 expression and signaling in the inflammatory infiltrates, is that, over time, there could be a progressive loss of BMP signaling in the inflamed stomach, leading to the development of a pro-oncogenic

environment, similar to that seen in the *H pylori*–infected noggin transgenic mice, that ultimately could be responsible for the development of dysplastic and neoplastic changes.

In conclusion, our study provides evidence for the existence of a mechanism based on the release of a mesenchymal peptide, BMP-4, that specifically activates a signaling pathway in the gastric epithelium that exerts inhibitory effects on the expression of proinflammatory mediators. These findings underscore the importance of BMP signaling in the regulation of gastric inflammation and epithelial homeostasis, providing new clues for a better understanding of the pathophysiological mechanisms that lead to the development of both dysplastic and neoplastic lesions in the stomach. Additional investigations and possible manipulations of the BMP signal transduction pathway therefore might offer novel opportunities for the treatment of gastric inflammation and carcinogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used in this paper

AID	activation-induced cytidine deaminase
BMP	bone morphogenetic protein
IL	interleukin

LPS	lipopolysaccharide
MIP-2	macrophage inflammatory protein-2
mRNA	messenger RNA
QRT-PCR	quantitative reverse-transcription polymerase chain reaction
SMA	smooth muscle antibody
STAT	signal transducer and activator of transcription
TFF2	trefoil factor 2
TG	transgenic
TNF	tumor necrosis factor
WT	wild-type

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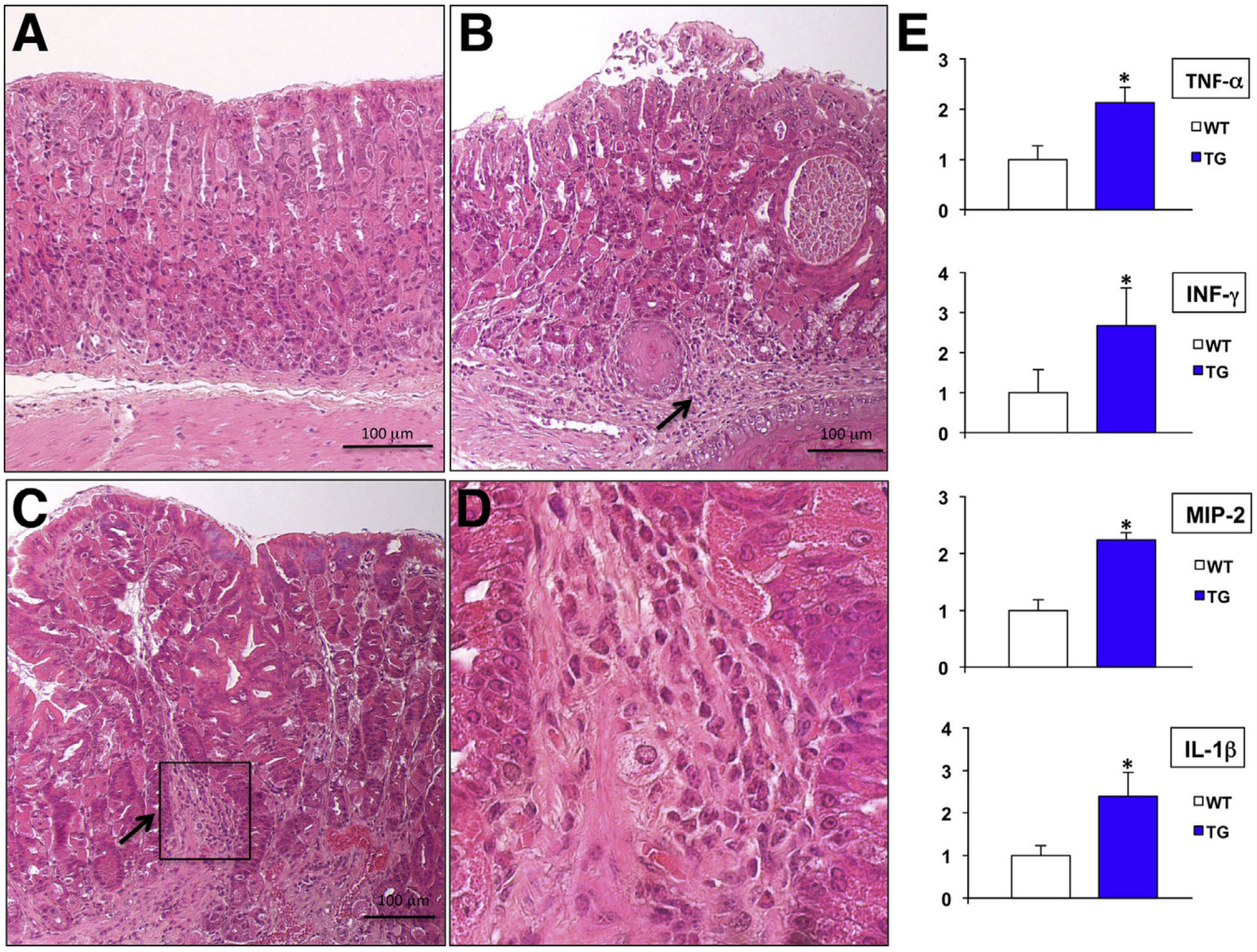

Figure 1.

Inflammation in noggin TG mice. Representative H&E-stained paraffin sections of the corpus of (*A*) 12-week old WT and (*B* and C) TG mice. *Arrows* point to inflammatory cells. (*D*) *Magnified window* depicting inflammatory cells. (*E*) TNF-α, IFN-γ, MIP-2, and IL-1β mRNA abundance in WT mice was compared with that detected in TG mice using QRT-PCR and displayed as a fold-increase over the non-TG (WT) negative controls. Values are shown as means ± standard error (n = 4). **P* < .05.

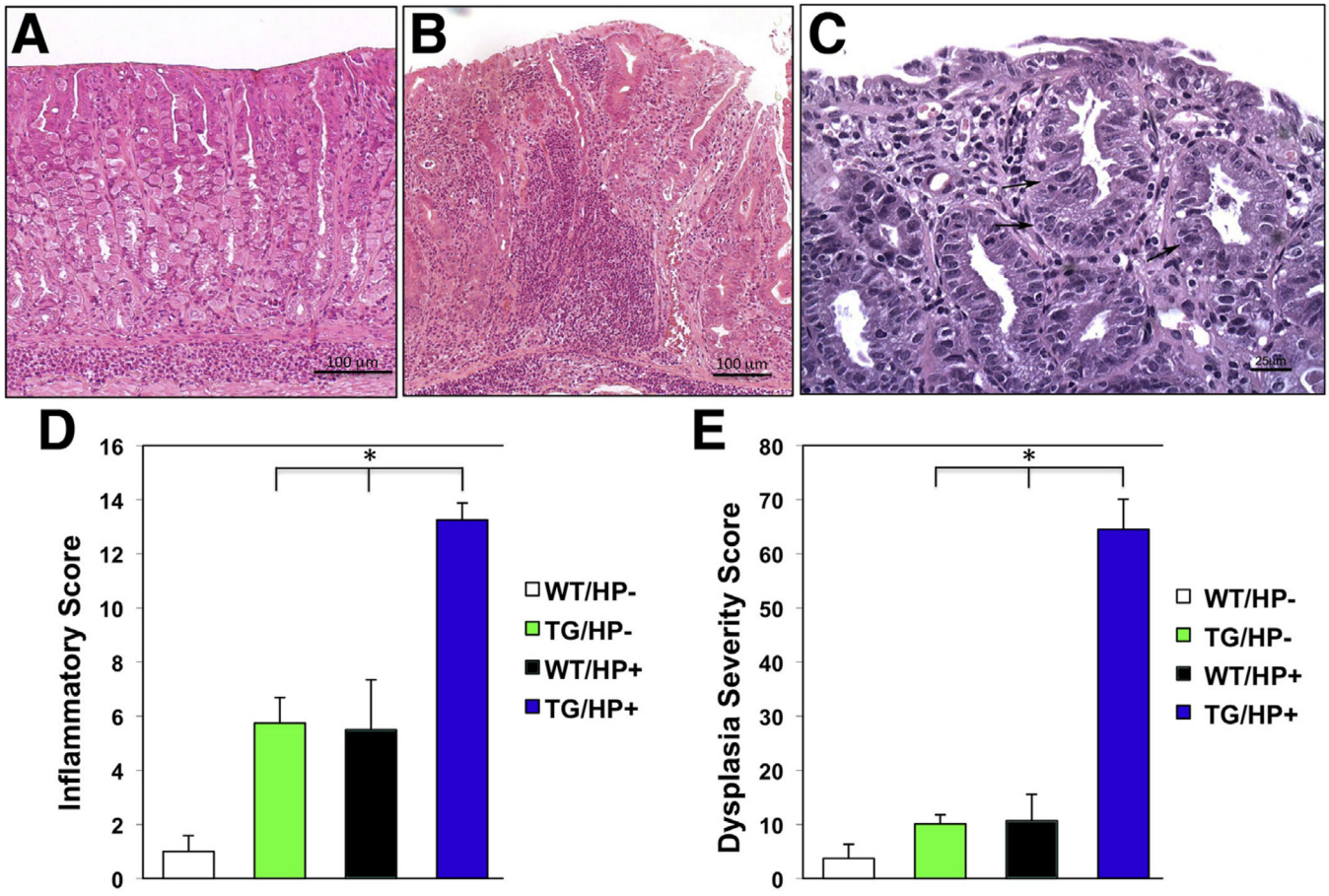

Figure 2.

Enhanced inflammation and accelerated dysplasia in the gastric epithelium of *Helicobacfer*-infected noggin TG mice. Representative H&E-stained gastric paraffin sections of the corpus of (*A*) *H pylori* (HP)-infected WT and (*B*) TG mice. (*C*) Dysplastic changes in *Helicobacter*-infected TG mice. *Arrows* point to areas of dysplastic epithelium. *Bars* represent the (*D*) inflammatory and (*E*) dysplasia severity scores calculated in both WT and TG mice in the (*D*) presence and absence of *H pylori* (HP). Values are shown as means ± standard error (n = 4). **P* < .05.

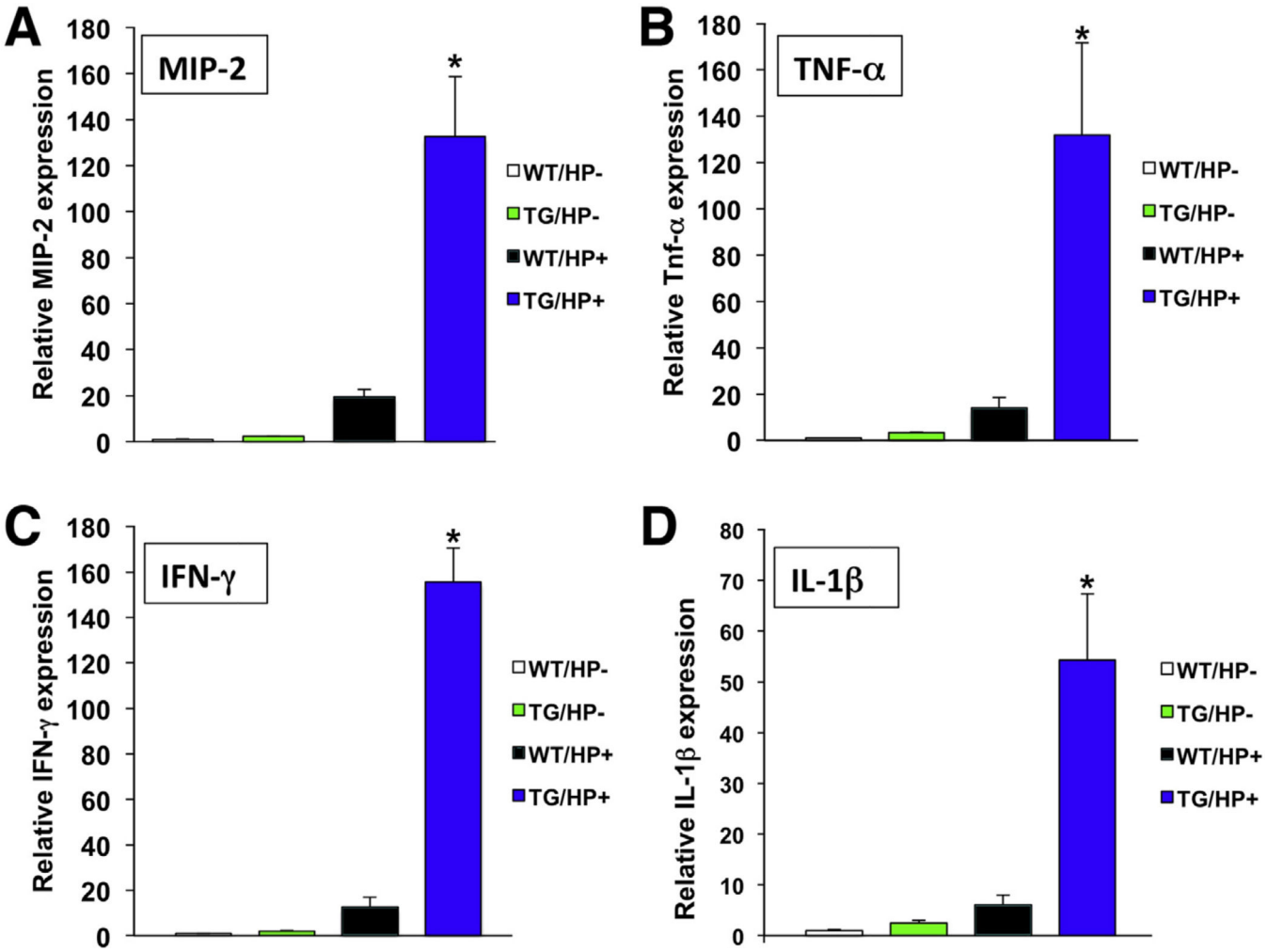

Figure 3.

Helicobacter infection increases the expression of proinflammatory cytokines in noggin TG mice. (*A*) MIP-2, (*B*) TNF-α, (*C*) IFN-γ, and (*D*) IL-1β mRNA signals in WT mice were compared with those detected in TG mice in the presence and absence of *H pylori* (HP) using QRT-PCR and displayed as a fold-increase over the WT-negative controls. Values are shown as means ± standard error (n = 4). **P* < .05.

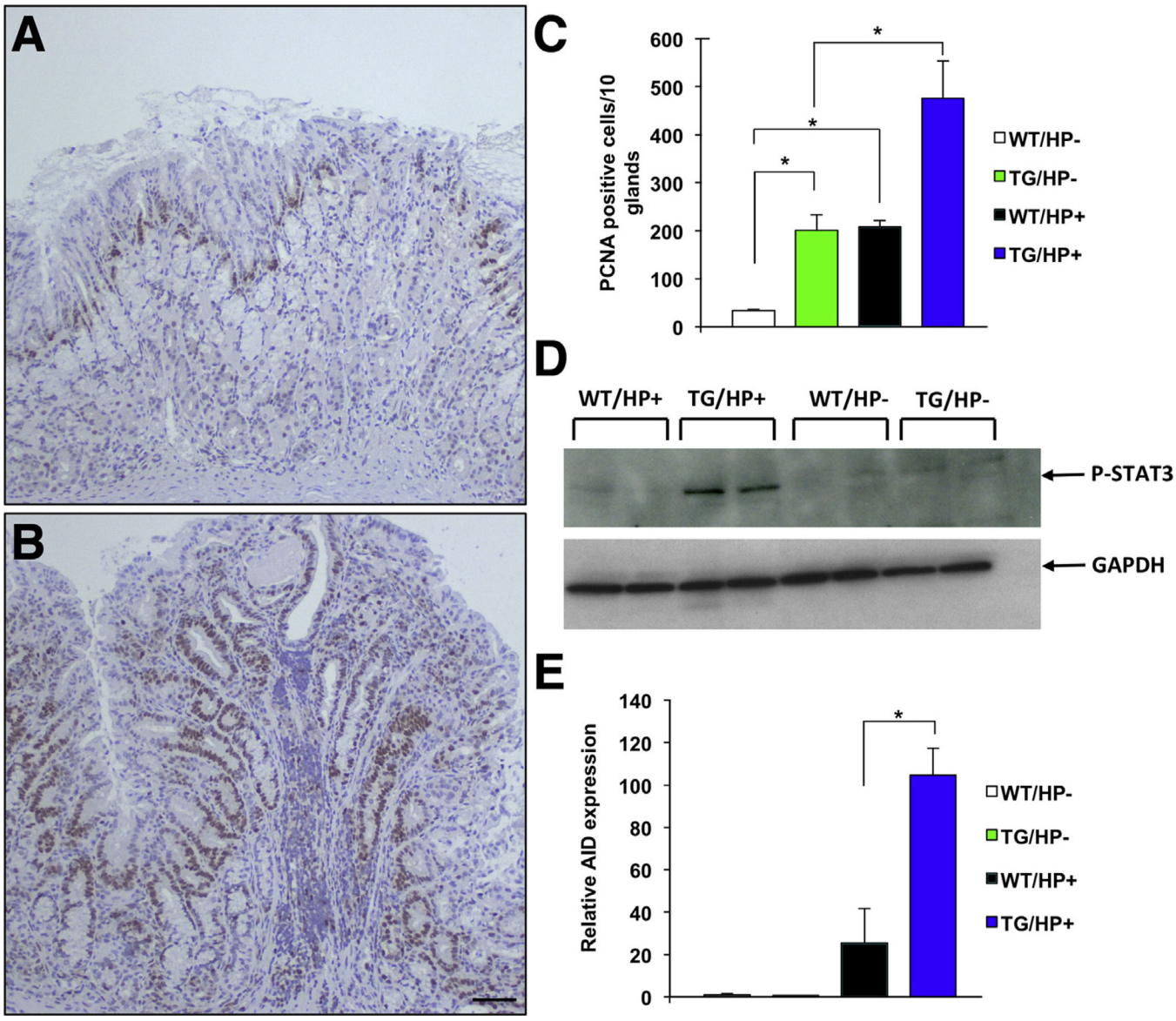

Figure 4.

Increased cell proliferation and expression of pro-oncogenic molecules in *Helicobacfer*-infected noggin TG mice. Gastric paraffin sections from (*A*) noninfected and (*B*) *H pylori* (HP)-infected TG mice were stained with anti–proliferating cell nuclear antigen (PCNA) antibodies. *Scale bar:* 50 μm. (*C*) Graph bars represent the number of PCNA-positive nuclei detected in WT and TG mice in the presence and absence of HP. Values are shown as means ± standard error (n = 4). **P* < .05. (*D*) Phosphorylation and activation of STAT3 in WT and TG mice in the presence and absence of *H pylori* was studied by Western blots using an anti-phospho-STAT3 antibody. (*E*) AID mRNA signals in WT mice were compared with those detected in TG mice in the presence and absence of HP using QRT-PCR and displayed as a fold-increase over the WT negative controls. Values are shown as means ± standard error (n = 4). **P* < .05. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

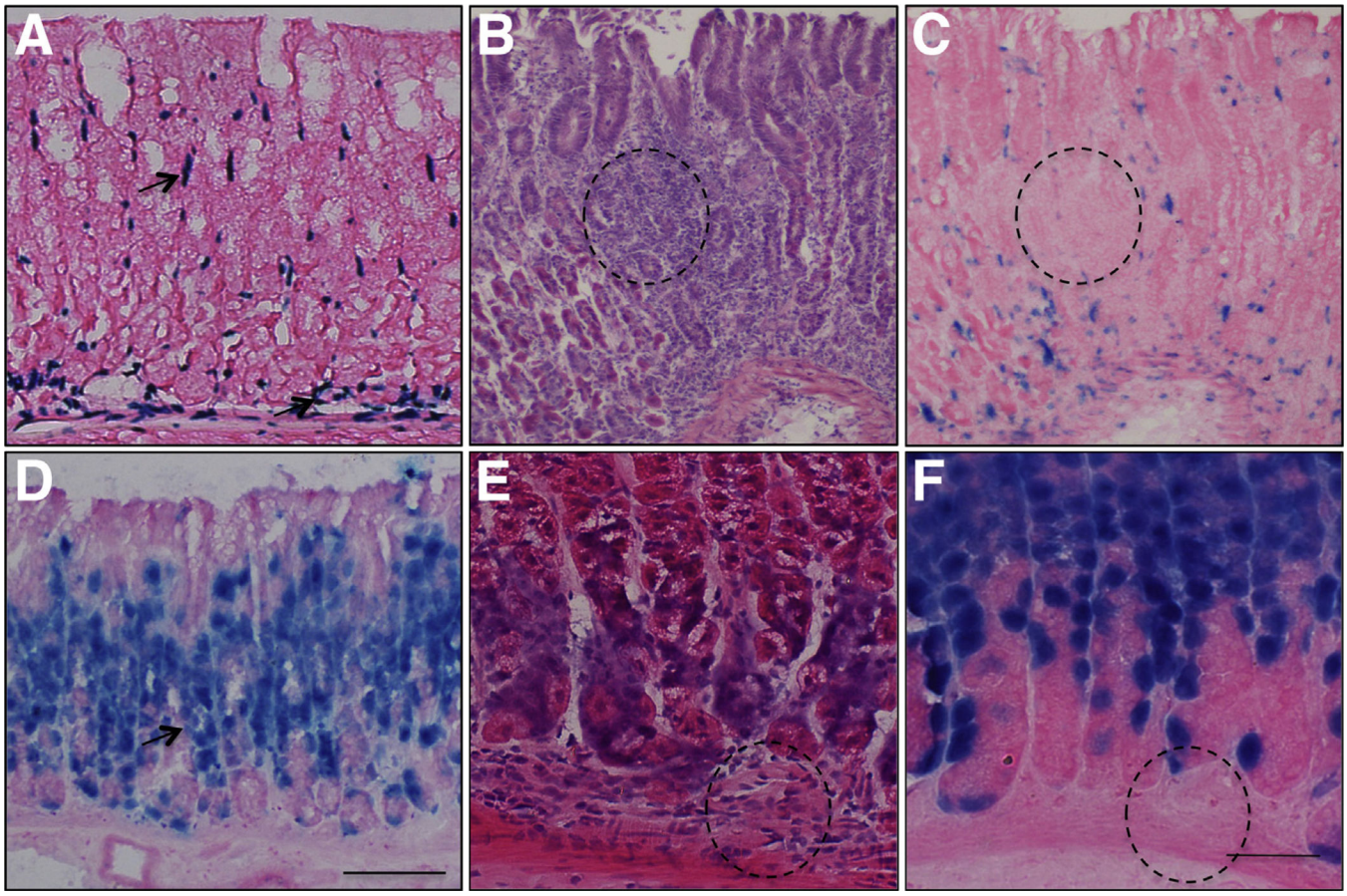

Figure 5.

Expression of BMP-4 and localization of BMP-activated signaling in gastric inflammation. (*A*) Gastric frozen sections from BMP-4$^{\beta\text{-gal}/+}$ mice stained with X- gal. (*B*) H&E and (*C*) corresponding X-gal–stained frozen sections from *H felis*–infected BMP-4$^{\beta\text{-gal}/+}$ mice. (*D*) Gastric frozen sections from BRE–β-gal mice stained with X-gal. (*E*) H&E- and (*F*) corresponding X-gal–stained frozen sections from *H felis*–infected BRE–β-gal mice. *Arrows* point to X-gal–stained (*A*) mesenchymal and (*D*) epithelial cells. (*B*, *C*, *E*, and *F*) *Dotted circles* mark matching areas of the sections with inflammatory infiltrates. *Scale bars:* (*A*–*D*) 100 μm, (*E* and *F*) 50 μm.

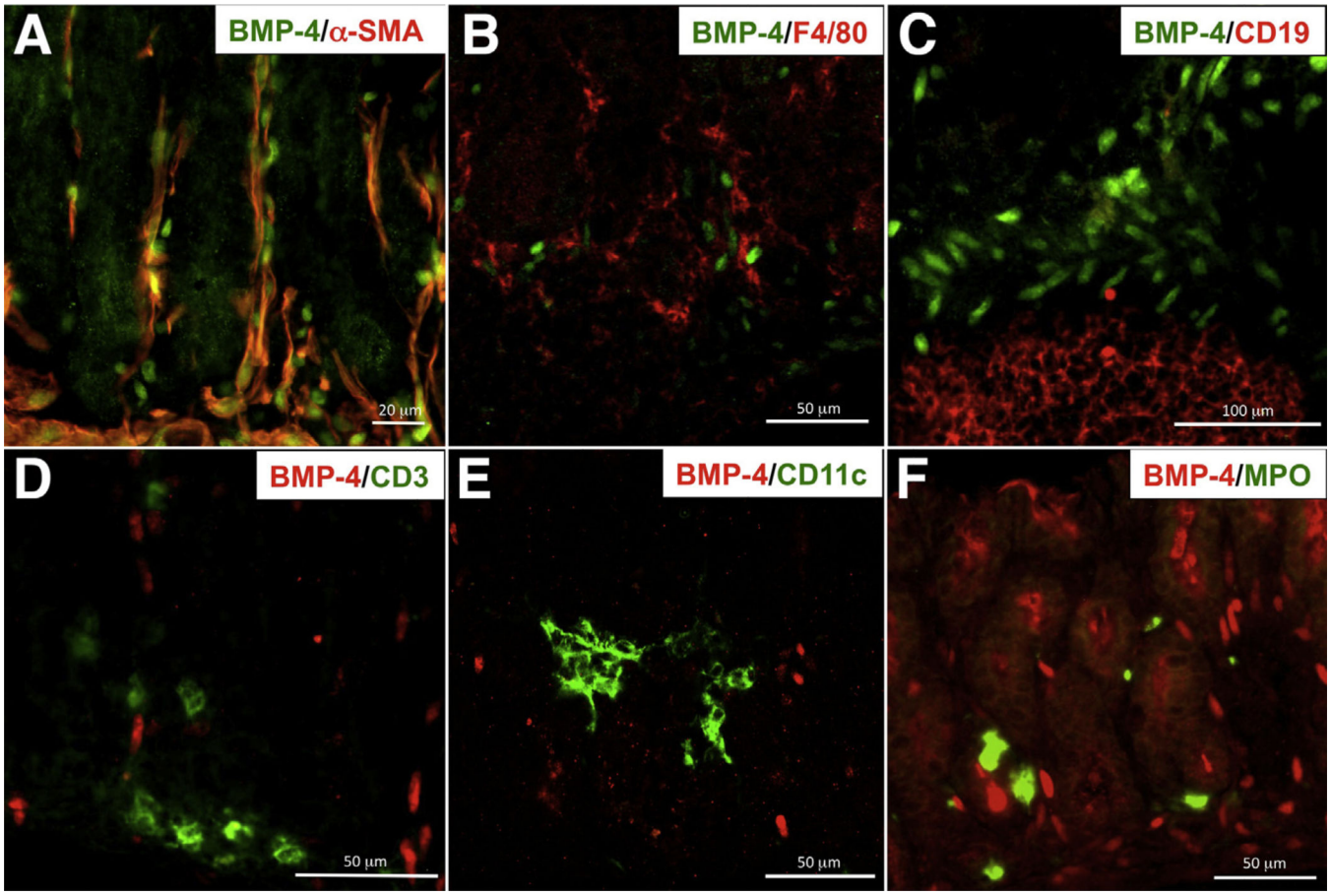

Figure 6.

Localization of BMP-4 in mesenchymal and inflammatory cells. (*A–C*) Gastric frozen sections from *H felis*-infected BMP-4$^{\beta\text{-gal}/+}$ mice were stained with anti–β-galactosidase primary antibodies and fluorescein isothiocyanate (FITC)-conjugated secondary antibodies (*green*), together with Cy3-conjugated anti-actin, (*A*) α-smooth muscle antibodies (*red*), (*B*) anti-F4/80, and (*C*) anti-CD19 primary antibodies, and Alexa 594–conjugated secondary antibodies (*red*). (*D–F*) Gastric frozen sections from *H felis*-infected BMP-4$^{\beta\text{-gal}/+}$ mice were stained anti–β-galactosidase primary antibodies and Alexa 555–conjugated secondary antibodies (*red*), together with (*D*) fluorescein isothiocyanate (FITC)-conjugated anti-CD3, (*E*) FITC-conjugated anti-CD11c, and (*F*) FITC-conjugated anti-myeloperoxidase (MPO) antibodies (*green*). Results similar to those depicted in the figure were observed in at least 3 other separate experiments.

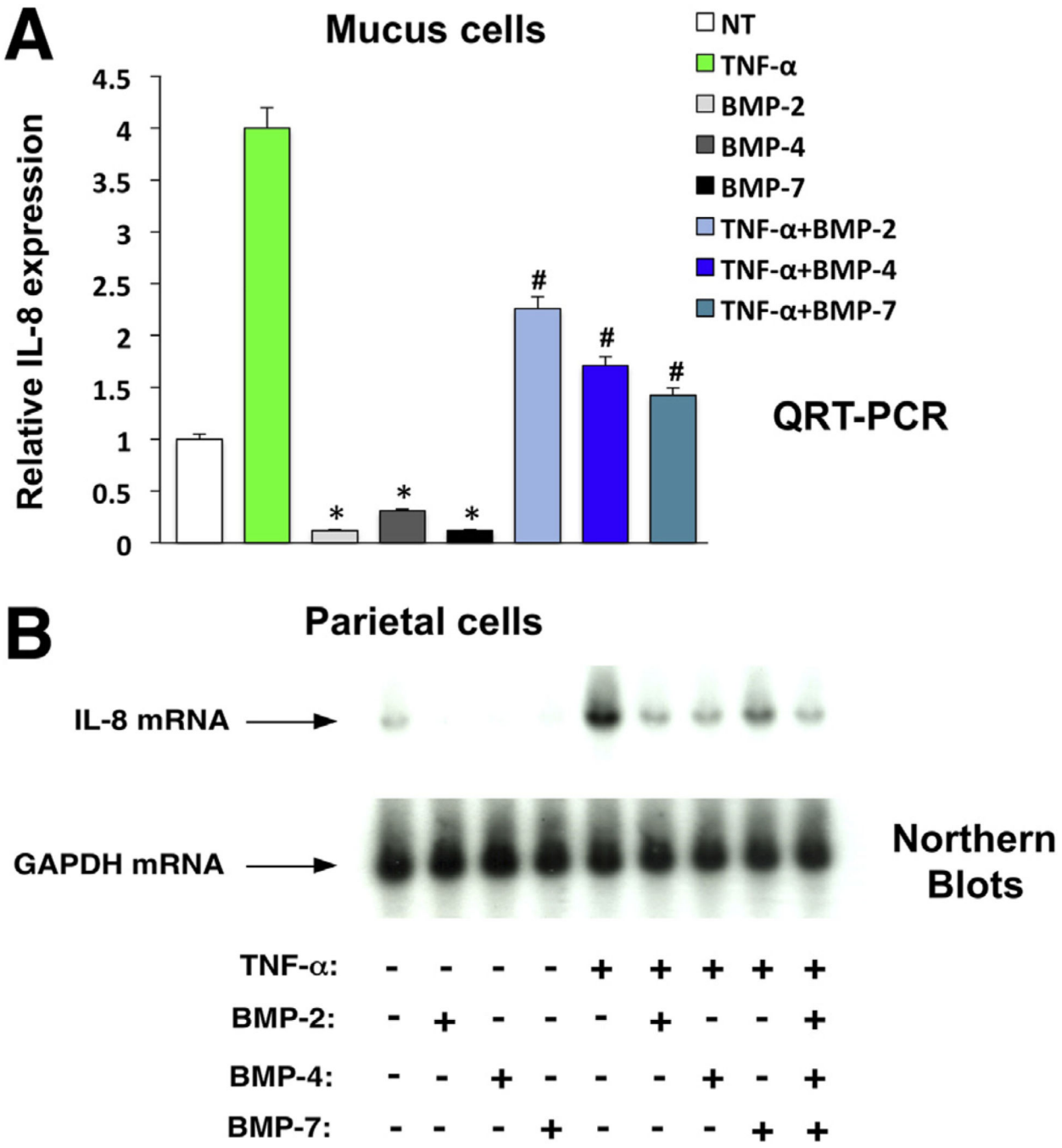

Figure 7.

Inhibition of basal and TNF-α–stimulated IL8 gene expression by bone morphogenetic proteins in isolated canine gastric epithelial cells. IL-8 mRNA abundance in (*A*) canine mucus and (*B*) parietal cells stimulated with TNF-α (10 ng/mL), in the presence and absence of BMP-2 (20 ng/mL), BMP-4 (20 ng/mL), and BMP-7 (20 ng/mL), alone or in combination, was measured by QRT-PCR and Northern blots, respectively. Values are

shown as means ± standard error (n = 3). *$P < .05$ and #$P < .05$ vs basal and TNF-α-stimulated IL8 gene expression, respectively.