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Production of Ectopic Gastric Intrinsic Factor in Gastric Mucosa of Humans with Chronic Gastritis

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Abstract

Background—Ectopic expression of gastric intrinsic factor (IF) has been described in rodent models of chronic gastritis.

Aims—The current study undertook to determine if ectopic IF was also present in chronic gastritis in humans and might identify the process of ectopic protein expression as part of the response to chronic injury.

Methods—Archived biopsies from mid-body, angularis and prepylorus of 9 patients with and without chronic gastritis and food-cobalamin malabsorption were examined in a blinded fashion by immunocytochemistry as were biopsies from 5 normal subjects. Cells with ectopic IF were further examined with antibodies against pepsin or with Griffonia simplicifolia II (GSII) to identify cells in the mucous neck cell compartment.

Results—Ectopic IF production in non-parietal cells was identified in cells that were H⁺,K⁺-ATPase-negative but IF-positive in 7 of the 9 patients (6/9 in the angularis and/or prepylorus biopsies and 1/9 only in the mid-body). These included 5 of the 6 *H. pylori*-infected patients and all 5 patients with severe food-cobalamin malabsorption. No normal control subjects demonstrated ectopic IF. The cells with ectopic IF were pepsinogen-positive peptic cells and were not GSII-positive. Expression was most extensive in patients and gastric regions with inflammation. In all but one sample, ectopic IF was observed near anatomical mucosal junctions, such as antral/body and prepylorus/duodenum junctions.

Conflict of interest

None of the authors have financial conflicts of interest in regard to this study.

Conclusions—These data in humans with and without gastritis are consistent with the hypothesis that local factors influence ectopic gastric IF expression, arising from either the anatomical location, the focal inflammation, or both.

Keywords

Gastritis; Intrinsic factor; Parietal cells; Chief cells

Introduction

Gastric mucosal cells in the body (zymogenic or oxyntic region) of the human stomach consist of five major cell types that derive from a common stem cell: surface mucous, mucous neck, parietal, chief (zymogenic), and enteroendocrine. In humans and most mammals, gastric intrinsic factor (IF), the protein responsible for receptor-mediated cobalamin absorption, is expressed in the acid-secreting parietal cell [1, 2]. Loss of acid secretion is a major contributor to food-cobalamin malabsorption [3], and loss of IF (pernicious anemia) creates malabsorption of all cobalamin, whether free or food-bound.

In the mouse and rat, IF is normally expressed in the zymogenic or chief cells instead of parietal cells as in humans [2, 4, 5], but 4–11% of isolated parietal cells in the rat have been found to also express IF ectopically (i.e., not in the chief or zymogenic cell line) [5]. In human mucosa obtained from transplant donors, too, individual clusters of chief cells and enteroendocrine cells were ectopically positive for IF [6]. Most of these clusters were found at the margins of the anatomical regions of the stomach, between cardia and body or between body and antrum.

These observations are consistent with the ability of cell lineages to alter their predetermined expression of proteins [7], perhaps mediated by local tissue factors (e.g., cytokines [8] or leukotrienes [9, 10], by maturation, response to injury, or both. The differentiation of gastric cell lineages has been explored most extensively in the mouse. The isthmus contains the proliferative cells, and upward moving cells differentiate into pit (foveolar cells). The cells that move downward from the isthmus form glands that are specialized in different parts of the stomach. In the fundus, some cells become mucus neck cells, and others differentiate into chief cells [11]. Parietal cells are found only in the fundic mucosa, and may also derive from preneck cell progenitors [12]. In the antrum, the downward moving cells become enteroendocrine cells and pyloric gland cells [11]. In the fundic region, the factors regulating differentiation to chief cells include the transcription factor *Mist1* [13]. The characteristic expression pattern of proteins can be disrupted in gastric cancers or by inflammatory stimuli, such as *H. pylori* [14].

Intestinal metaplasia and spasmolytic polypeptide-expressing metaplasia (SPEM) have been reported in precancerous human stomachs [15]. Chief cells transdifferentiate in the gastric fundus in mice into spasmolytic polypeptide-expressing metaplasia (SPEM), resembling deep antral gland cells and expressing Trefoil Factor 2 (TFF2). This process occurs in the presence of chronic inflammation from *H. pylori* infection in mice. Peptic cells in human stomach, identified by the presence of pepsinogen, have been identified as chief and mucous neck cells in the fundus, in pyloric glands in the antrum, and in cardiac glands [16].

Heterotopic and metaplastic cells also contain pepsinogens, similar to the normal cells. Antral glands are enriched for the pepsinogen-II isomer, whereas fundic mucous neck cells contain mostly pepsinogen-I [17]. This peptide distribution has been confirmed by the distribution of mRNAs [18].

Parietal cells have been shown to produce peptides and factors that might regulate differentiation within gastric glands, in addition to regulating acid production [19]. Much of the cell transcriptome is dedicated to cellular energy metabolism and mitochondrial function, consistent with its role in acid production. However, parietal cells also express and secrete growth factors (heparin-binding epidermal growth factor and insulin-like growth factor binding protein-2), a PTH-like peptide, and VEGFb. In humans, *H. pylori* infection causes inflammation mainly of the antrum, but it can proceed to the corpus to produce multifocal atrophic gastritis [20].

Because of all these observations, gastric tissue specimens from a well-characterized series of patients with various grades and types of chronic gastritis from an earlier study of gastric histology and function in relation to food-cobalamin malabsorption [21] were examined for the presence of ectopic IF. The purpose was to (1) confirm in patients with chronic gastritis the ectopic IF findings seen previously in animal models and in transplant donors, and (2) examine if inflammatory or atrophic gastritis, or both, influenced the expression of IF in cells other than parietal cells in humans.

Methods

Tissue Specimens

Gastric biopsy material was selected from patients with and without chronic gastritis who had been previously studied in a survey of gastric and cobalamin status, which had been approved by the Institutional Review Board and for which signed informed consent had been obtained [21]. These patients had been selected from an elderly population with low or normal serum cobalamin levels, whose cobalamin absorption status had been established, including by egg yolk-cobalamin absorption testing for food-cobalamin malabsorption (which affects patients with gastritis and other gastric disorders but does not involve IF deficiency), and who volunteered for an endoscopic examination. In all cases, the diagnosis of pernicious anemia (i.e., malabsorption caused by lack of IF) had been excluded [21]. In that earlier study, the biopsies had been obtained during endoscopy with a large-capacity pinch biopsy forceps from the pre-pyloric region (near the antral/pyloric junction), from the greater curve (mid-body and 3 cm distal to it) where the folds are thickest, and from the angularis, near the antral/body junction. All biopsies were mounted with the luminal surface up on a plastic mesh and fixed in Bouin's solution for 2–6 h before transfer to 70% alcohol. Slides containing 4–6 serial sections at 4 µm were prepared after processing and paraffin embedding, and were stored at room temperature. Gastric biopsy specimens from 9 of the original 19 patients were selected for the present study if unstained slides containing serial sections were available. Availability of adjacent sections was essential for identifying the morphology of cells that stained positively on immunohistochemical analysis.

Biopsy specimens were coded, mixed, and sent to the investigators at Washington University, where they were processed for immunocytochemistry and read in blinded fashion. Values for gastritis index, atrophy score, cobalamin absorption findings, gastric secretory findings, and *H. pylori* status were obtained from the original report of these patients [21]. The patients' demographic and diagnostic information is summarized in Table 1. The patients' numerical designations in the original study [21] were retained in the present report to preserve easy comparison to the earlier data, and they explain the nonconsecutive identifying patient numbers in the present text and Tables. All patients had significant corpus and antral gastritis, except case 12, who had normal cobalamin absorption, negligible inflammation of the corpus only, and negative breath test for *H. pylori*. The other cases fit the revised Sydney classification of multifocal atrophic gastritis [22]. Case 3 had very elevated serum gastrin, and was the only subject with positive intrinsic factor antibodies. However, the Schilling test for free cobalamin absorption was normal and he had IF activity, albeit very reduced, in his gastric juice, and so pernicious anemia was excluded.

Biopsies were also examined from five additional patients with normal gastric mucosa who were recruited from those with obscure gastrointestinal bleeding and undergoing upper endoscopy at Washington University. Their biopsies were obtained and processed according to a protocol similar to that noted above, although gastric acid and IF secretion and absorption were not measured. All these patients were negative for *H. pylori* on biopsy and urease testing.

Immunocytochemistry—Immunostaining was performed using the avidin-biotin-peroxidase complex method as reported previously, followed by counterstaining with hematoxylin [5, 23]. Endogenous peroxidase was quenched by treatment of the slides with 1% H₂O₂ for 20 min. The antibody against human IF was polyclonal and raised in rabbit against recombinant human IF produced in baculovirus-infected Sf9 cells [6]. This antibody has been shown to be specific for IF, as staining was blocked by addition of recombinant human IF [23]. Two additional antibodies were used to test the cells that were IF-positive but were not found in regions where parietal cells typically occur. Rabbit antibody against another parietal cell marker, the β -subunit of rat H⁺/K⁺-ATPase (amino acids 2–33) was generously provided by Michael J. Caplan (Yale University). This antibody had previously been shown to be equally robust in both murine and rat gastric sections [23], and was equally so in preliminary experiments using paraffin sections of gastric mucosa taken by endoscopic biopsy from the oxyntic region of the human stomach. Rooster anti-human pepsin antibody (raised against pepsin purified from human gastric juice), which serves as the marker for the peptic/chief cells, was generously provided by Norma A. Metheny (St. Louis University School of Medicine). This antibody has been used to develop an immunoassay for human pepsin [24]. All antisera were used at a dilution of 1:200.

One-half of each slide was stained with each antibody, so that at least four serial sections were stained, two each using antibody raised against IF and H⁺/K⁺-ATPase, or against IF and pepsin. One such section from all three mucosal regions (angularis, midbody, and prepylorus) from each patient was stained for IF and H⁺/K⁺-ATPase. All slides were observed and the positions of IF-positive, H⁺/K⁺-ATPase-negative cell clusters were recorded using

the micrometer on the microscope. Sections with no such clusters were graded as 0, those with only 1–3 such clusters per section were graded as 1+, and sections with >3 clusters/section were graded 2+. To demonstrate that those cells that stained positively for IF but not for H⁺/K⁺-ATPase were indeed chief cells rather than, for example, parietal cells that failed to demonstrate H⁺/K⁺-ATPase reactivity, several such specimens were chosen for labeling for both IF and pepsin, depending on the availability of unstained adjacent serial sections.

Double-labeled immunofluorescence was performed as described previously [25], except that different antisera were used. After overnight incubation with the first primary antibody (rabbit anti-human IF) at 4°C, goat anti-rabbit IgG secondary antibody labeled for green fluorescence with FITC (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) was added for 1 h at room temperature. The second primary antibody (rooster anti-human pepsin) and its secondary antibody, donkey anti-chicken IgG labeled for red fluorescence with Cy3 (Jackson ImmunoResearch Laboratories), were added for 1 h at room temperature. The secondary antisera were both used at 1:200 dilution. A krypton-argon laser was used with epifluorescence filter sets designed for Texas Red (SR01 and Cy3) and fluorescein (FITC-VVA and FMI-43), and the slides were viewed with a Zeiss 63 × 1.4 numerical aperture and planapochromat objective.

In some studies, double labeling was performed with fluorescent conjugates of lectin from *Griffonia simplicifolia* (GSII) that has been used to identify the mucous neck cell region by detecting the expression of terminal *N*-acetyl-D-glucosamine residues in a wide variety of tissue and cell types [26]. This lectin also recognizes *N*-acetyl-D-glucosamine at the end of *O*-glycosylated sugar chains. It labels mucous neck and deep antral cells in normal stomach, and SPEM in the *H. pylori*-infected stomach, at least in mice. Primary antibodies identifying human IF were used with a second antibody, goat-anti-rabbit IgG labeled for green fluorescence with FITC (Jackson ImmunoResearch Laboratories). The GSII, Alexa Fluor[®]594 conjugate (L-21416), a superior spectral analog of Texas Red[®] (Molecular Probes, Eugene, OR, USA), was then used at a dilution of 1:500 with overnight incubation at 4°C. The double-labeled slides were then incubated at room temperature for 1 h, washed, and covered using VectaShield mounting medium (Vector Laboratories, Burlingame, CA, USA). Images were taken using a ×10 or ×40 objective using a software-controlled (LeicaFW 4000) digital camera (Leica DFC 350FX) attached to microscope (Leica DM 4000B). The contrast adjustment superimposition was done with Adobe Photoshop 6.0 for PC.

Results

All biopsies taken from the five subjects from Washington University with normal gastric mucosa showed the presence of IF-positive cells in the body of the stomach, and in biopsies taken from the region of the angularis. All the IF-positive cells in one section were also positive for H⁺/K⁺-ATPase in adjacent sections (Fig. 1). Small clusters of IF-positive cells have been reported by us in areas of a normal stomach that did not contain parietal cells (cardia, antrum) when the entire stomach was sectioned and examined [6]. However, in the much smaller sampling from endoscopic mucosal biopsies in these five normal subjects, no

IF-positive cells were found in gastric glands in the angularis and prepylorus, regions that were not also positive for H/K ATPase, the marker for mature parietal cells.

Seven of the nine studied patients, including all 5 with severe food-cobalamin malabsorption, showed cells with ectopic IF in biopsies. Six of 8 were positive in biopsies taken from the angularis and prepylorus, although occasional patients demonstrated changes in only one of the two sites (Table 2). The 9th patient (#12), who had negligible inflammation limited to the corpus and had neither *H. pylori* infection nor cobalamin malabsorption, showed ectopic IF only in the mid-body. The most widespread distribution of cells with ectopic IF occurred in the *H. pylori*-infected patients with extensive gastric inflammation and severe food-cobalamin malabsorption (cases 4–6). Cases 2, 3, and 16 had the most prominent atrophy in the biopsies with rather less inflammatory changes, and showed less prominent ectopic IF. Ectopic IF was also seen to a lesser extent in the prepylorus and angularis of patient 18 who, despite the presence of *H. pylori* infection and inflammatory as well as atrophic changes, had normal cobalamin absorption.

Among the biopsies examined from the 9 patients who participated in the earlier survey [21], cells that were negative for H⁺/K⁺-ATPase but stained positively for IF (ie, ectopic IF) were seen most frequently in sites where inflammation was most prominent, at both ends of the antrum (angularis and prepyloric regions). In patients 5 and 16, in whom such cells were seen in the mid-body of the stomach, they were also in association with areas of inflammation (Table 2). Only one subject (case 7) showed no cells with ectopic IF in any of the 3 biopsy samples. Case 7 had mild food cobalamin malabsorption and was positive for *H. pylori*, but had quite limited corpus gastritis and inflammation.

Characterization of Cells with Ectopic IF as Peptic Cells

The cells expressing ectopic IF were most easily recognized in sections from the angularis and prepyloric regions, where they occurred in clusters, as demonstrated by sections from case 4 (Fig. 2a). The ectopic cells demonstrated cell shapes that were not recognizable as parietal cells, but had the shape of less triangular cells, as seen in the cluster of cells on the left hand side of Fig. 2a, b (see lower arrow). These cells were not positive for H⁺/K⁺-ATPase in an adjacent section (Fig. 2b). No portion of the IF-positive clusters from the angularis or prepyloric region stained positively for H⁺/K⁺-ATPase in adjacent sections. Occasionally, IF-positive cells were seen within mucous glands, but always near the cluster of non-mucous glands that produced IF (Fig. 2a, b, arrowhead). Because sections from the archived tissue of the previous study were limited, this preliminary single labeled study was used to screen for regions suitable for double labeling. Thus, double-labeled studies were not performed in this first set of experiments.

On the other hand, within the mid-body of most subjects, the cells that were positive for IF coincided with those that were positive for H⁺/K⁺-ATPase, and no clusters of cells producing ectopic IF could be identified (Fig. 2c, d, from case 4). This is identical with the findings seen in the angularis and mid-body biopsies from the five control subjects with normal gastric mucosa (Fig. 1).

Double-labeled immunofluorescence was used next to determine whether the ectopic-IF cell clusters were indeed one of the types of peptic cells, as judged by co-expression of pepsin. Cell clusters were first identified as negative for H^+/K^+ -ATPase when stained on an adjacent section, the screening test used to select sections for double-labeled analysis (see Fig. 2a, b as an example). Figure 3a–c shows the results of double labeling from an angularis biopsy of subject 3. All these cells stained for both IF and pepsinogen, consistent with a peptic cell-type that elaborates ectopic IF.

Shown for comparison is the basal region of a mid-body biopsy from another patient (#6), in which IF and pepsinogen were expressed mostly on different cells (Fig. 3d–f). Most of the IF-producing cells can be identified by shape as either larger and/or rounded, consistent with parietal cells (see Fig. 2a). However, in the mid-body of the stomach, most cells expressed IF and pepsinogen in their appropriate separate cells (parietal and chief cells, respectively). Figure 3d–f shows only a few cells that contain both IF and pepsinogen (see arrows). These infrequent double-labeled cells were not appreciated in the single-antibody examination of this section. Individual cells containing both IF and pepsinogen were seen in some, but not all, mid-body sections from other patients (cases 2 and 16).

Lack of Mucous Neck Zone Localization for the Ectopic IF Cell Clusters

The mucous neck cell zone consists of mostly postmitotic, relatively long-lived, mucus-secreting cells from which are derived the more fully differentiated cells in the surface and basal regions of the gastric glands [11]. A further double-labeled experiment was performed to determine whether the ectopic cells containing pepsinogen and IF resulted from expansion of this mucous neck cell compartment, or resulted from up-regulated expression of IF in well-differentiated chief cells, chief cell precursors, or other peptic cells in the cardia or prepyloric regions. Cells from the mucous neck zone of the gastric gland have been identified by the GSII lectin-positive mucous neck cells that increase during inflammation [27]. Adjacent sections from the same tissue block were taken from cases 6 and 18 that had shown ectopic cells in the antral mucosa near the angularis (Table 2). For comparison and to demonstrate the cells in the normal mucous neck zone in the upper 1/3 of the gastric glands, sections were taken from the gastric corpus (mid-body) of all cases that showed no cells producing ectopic IF in that region (case 12 is used as representative of these cases). In case 12, the GSII-positive cells were limited to the mid/lower-gland region of the gastric corpus, and appear to be homogeneous and not too abundant (data not shown). The IF-positive cells were abundant within the mucous neck zone, as expected, but were also distributed toward the more basal portion of the mucosa of the gastric body. No GSII-positive cells that also expressed IF were identified. In contrast, cases 6 and 18 with ectopic IF-expressing cells in biopsies of antral mucosa from the angularis (the junction between the gastric body and fundus) had fairly marked gastritis ratings (Table 2). In both cases 6 and 18, the mucous neck zone was markedly expanded as expected from the presence of gastritis (Fig. 4 shows results for case 18). Intense inflammation can be noted (Fig. 4a, b) surrounding the glands with ectopic IF expression, and the cells that express IF are found on the upper portion of the GSII staining mucous neck zone. The regions examined were within a few sections (2 μ m thick each) from the regions used to demonstrate cells that expressed both IF and pepsinogen (Fig. 3), yet in none of the sections from either case 6 or 18 or in two others with

ectopic IF (data not shown) were cells found that were positive for both GSII (red) and IF (green). These results are consistent with the production of ectopic IF within cells that had already committed to differentiate into the peptic cell lineages.

Discussion

This study shows that cell clusters expressing IF ectopically (not in parietal cells) occur in the presence of gastritis in the human stomach. This finding is consistent with models of chronic gastritis in rodents in which IF is expressed in cells other than chief cells, the default cell for IF expression in rodents [23]. The samples were from patients with well-characterized gastric function and histology, but it should be noted that the original study from which these samples were selected was for the purpose of gastric characterization in relation to the presence or absence of food-cobalamin malabsorption [21]. Therefore, its control subjects were selected purposely for absence of malabsorption rather than for normal gastric histology. That report had studied patients with food-cobalamin malabsorption, a common condition defined by the inability to detach ingested cobalamin from its food binders and absorb it, even though IF is secreted in adequate amounts and functions normally and the Schilling test gives normal results [3]. The results of that study showed that cobalamin malabsorption did not arise solely from atrophic gastritis and achlorhydria, but might also reflect other gastric processes, including inflammatory gastritis such as in gastric infection with *H. pylori* [21].

The strengths of this study include the translation of findings in rodent gastritis models to human gastritis itself, and the demonstration that ectopic IF production appears to occur in cells that have already started along the pathway of cellular differentiation. There are, however, potential weaknesses that the reader should note. First, archived biopsy slides were used from a study of human gastritis designed for a different purpose. This has produced a group of patients who were heterogeneous for the extent of ectopic IF production. Second, although some control subjects were added, these were not part of the original design for the gastritis subjects. Thus, although ectopic IF is clearly more prevalent in gastritis patients, it is possible that with a larger group of control subjects an occasional cell producing IF ectopically might have been found. Third, the expression of ectopic IF was scored semiquantitatively, and not by extensive counting of complete sections. However, the purpose of the study was to demonstrate that the phenomenon of ectopic IF production did indeed occur in human gastritis. Future prospective studies will be needed to determine the precise extent of the phenomenon.

The limited number of subjects with truly normal gastric status in the present report, namely subject 12 and the five added control subjects, thus allow only limited comparisons of normal versus abnormal stomachs outside the scope of food-cobalamin malabsorption. Nevertheless, they and our subjects with different types of gastritis provide strong evidence for the expression of ectopic IF with gastritis and offer contrasts to patients with more normal gastric morphology. The current study probably provides a more realistic indication of the ability to find ectopic IF clusters in human stomach, as the sampling was done from endoscopic mucosal biopsies, rather than sampling the entire stomach, the method first used to find cells that expressed IF ectopically [6].

It appeared that there were no clusters of ectopic IF-producing cells in biopsies taken from the mid-body of the stomach. However, that region of the stomach also has the largest number of parietal and chief cells, so that single cells or small clusters of ectopic IF-producing cells might be harder to detect there (see Fig. 3d–f). Clusters of cells expressing ectopic IF were most evident in the prepyloric region and at the angularis near the junction of the body and antrum. This location for IF-expressing cells is in contrast with the distribution of normal parietal cells in the human antrum, which occur most often just proximal to the gastroduodenal junction but are not often found at the junction of the antrum and body [28]. These parietal cells do not need to be related to inflammation and metaplasia, because parietal cells have been found in the antrum of newborns and infants. These parietal cells exist in the prepyloric region of fetal stomachs but largely disappear during the third trimester of pregnancy and occur in only small numbers in the newborn's stomach [29, 30]. It is not clear whether the mechanism that produces parietal cells transiently in the newborn is related to that which leads to ectopic IF expression in the adult stomach with gastritis.

Ectopic peptic (or chief) cells have not been demonstrated in normal fetal stomachs in the region of the angularis or prepyloric region. Peptic cells do occur in normal gastric glands in human antral mucosa, as well as in metaplastic mucosa [16]. Pepsinogen is also found in the mucous neck cell zone in the fundus, but not in that zone in the antrum [16–18]. This finding is consistent with our observations of peptic cells that express IF in the upper third of the crypts in biopsies taken from the angularis. Because these IF-positive cell clusters are found unassociated with the mucous neck cell zone, it seems unlikely that they might be produced from stem cells, but rather from postmitotic cells that already might be partially moving down the pathway toward mature peptic cells. This movement seems directed by inflammatory mediators especially in the junctional regions of the stomach. It is not clear why parietal cells that have occurred normally in the antrum are not preferentially produced to express IF when inflammation is present. On the other hand, the clusters of cells that produce IF ectopically appear to be peptic cells, as they express pepsinogen and not H^+ , K^+ -ATPase.

Production of cell lineages in the mucous neck cell zone of the stomach is interrelated, as ablation of a differentiated cell line (e.g., parietal cells in mice) can lead to loss of all other cell types, including chief cells and enteroendocrine cells [31]. The role of parietal cells may be mediated by an ordered adherens junction network that also allows for migration of chief cells from the mucous cell zone to the base of the gastric glands [32]. In such a case, the primary effect of inflammation may occur in that zone, altering maturation of all cell lineages, as well as their gene expression. In the present study, altered IF expression was noted in a differentiated cell lineage, the peptic cell, and this change was seen most often in proximity to the mucous neck zone.

Figure 4 demonstrates that, despite expansion of the mucous neck cell population in the angularis, the IF-expressing cells did not express a mucous neck cell marker, GSII. This finding is consistent with the fact that in gastrin $-/-$ mice with inflammation, interferon-gamma treatment was associated with the production of increased numbers of mucous neck cells [27]. The finding of expanded mucous neck cells in biopsies of patients with chronic gastritis is consistent with the hypothesis that local concentrations of cytokines may be

inducing IF ectopically in pre-existing cells of peptic lineage. In the mouse model, however, the expanded mucous neck cell zone did not express chief cell markers. Thus, other factors or different cytokines may be important in the generation of the findings in human stomachs with chronic gastritis. Moreover, the origin of the peptic cells that produce these factors is not certain. The inflammatory infiltrate appears to be responsible for some secreted factors, because ectopic IF is seen only in glands with localized inflammation [23]. In response to *H. pylori* infection, transcriptomic profiling of laser captured regions of human gastric biopsies reveals pro-inflammatory and mucosal defense responses that are different in the three major cell types, parietal, chief, and mucus neck cells [33]. This finding is consistent with local production of specific cytokines when gastritis is present.

The distribution of cell clusters to junctional regions differs from the findings in experimental gastritis in animals, in which ectopic IF was detected largely in the midbody of the stomach [23]. In the animal studies, however, the timing of the biopsy could be controlled to maximize the degree of gastritis, and the entire stomach could be sampled. Moreover, some glands were inflamed next to normal glands, making it easier to detect ectopic IF in the mid-body. In human biopsies, it may have been easier to detect clusters of ectopic IF in junctional regions because of the relative absence of oxyntic glands. Moreover, many of the patients in this series were *H. pylori*-positive, a condition in which antral gastritis predominates.

When the 5'-untranscribed portion of the mouse IF gene (from -1,029 to +55 bp) was linked to human growth hormone and delivered as a transgene, IF was produced ectopically in parietal cells rather than the chief cells that normally express IF in normal mice [4]. This finding suggests that there are silencer regions that could be regulated to deliver the gene to its proper cellular destination. Chronic inflammation is one trigger that appears to be able to create ectopic foci of IF-producing cells, and to do so in humans in cells with the phenotype of peptic cells (pepsinogen-producing).

No regions of metaplasia were seen in the current biopsy specimens, so it is not possible to know whether ectopic IF might function as a biomarker for this process. It is clear that intestinal metaplasia and diffuse types of gastric cancer are preceded by inflammation, for which *H. pylori* infection is one cause [34]. The risk of cancer appears to be related to the degree of atrophy, and the available biomarkers track the degree of atrophy [35]. The present series is too small to prove an inverse relationship between the degree of atrophy and the presence or location of ectopic IF, but 4 of the 5 tissue sampling sites showing the most severe (grade 4) atrophy had no ectopic IF, whereas all 3 sites with the highest (grade 2+) ectopic IF had gastric atrophy scores of 2.0 or less (Table 2). Regarding inflammation, only 3 of 12 sites with no ectopic IF (score = 0) had gastritis grades >4.0, whereas 2 of the 3 sites with 2+ ectopic IF scores had gastritis grades of 7.0 or greater, suggesting a direct relationship. We conclude that the expression of IF in peptic cells may be due in part to local tissue factors.

An additional, novel, and non-exclusionary possibility may be raised for future consideration to add to the influence of local tissue factors. Although it was not directly addressed in this report, incipient cobalamin deficiency caused by diminished absorption

could theoretically produce a signal to redress the cobalamin balance by increasing IF production by any or all cells possible, whether peptic or residual parietal cells. This subclinical deficiency might have been present in many of our subjects with malabsorption of food-bound cobalamin, in which IF production was not abrogated. The extent of ectopic IF expression appeared to be greater in patients with severe cobalamin malabsorption than in those with normal absorption, or in the lone patient whose food-cobalamin absorption was only mildly impaired (Table 2). Whatever the mechanism of this ectopic expression, the potential of mature cells to alter their program of gene expression is clear. However, the clinical significance of this expression is unknown.

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Fig. 1. Immunohistochemistry in patients without gastritis, showing co-localization of IF and H/K ATPase expression in adjacent 4- μ m sections. Sections from one of the control subjects without gastritis are shown from the region of the angularis (**a, b**) and the midbody (**c, d**). Sections were stained using antibody against IF (**a, c**) or against H⁺,K⁺-ATPase (**b, d**). Note the lack of inflammatory infiltrate in the lamina propria of all sections. Staining was carried out as described in “Methods” and counterstained with hematoxylin. The time of staining was prolonged to detect any cells weakly positive for one of the antigens, thus creating some background staining. Magnification using $\times 20$ objective

Fig. 2.

Immunocytochemistry identifying clusters of cells that express intrinsic factor (*IF*) ectopically, defined as cells not expressing H^+,K^+ -ATPase. Sections were obtained from biopsies of subject 4 from the region of the angularis (**a, b**) and the mid-body (**c, d**). Sections were stained using antibody against IF (**a, c**) or against H^+,K^+ -ATPase (**b, d**). Note the significant inflammatory infiltrate in the lamina propria of (**a, b**). Staining was carried out as described in “Methods” and counterstained with hematoxylin. The *arrows* in (**a, b**) identify individual cells in glandular structures that are negative for H^+,K^+ -ATPase (**b**). The *arrowhead* in (**a, b**) identifies an IF-positive, H^+,K^+ -ATPase-negative cell in the adjacent mucous gland. The anti- H^+,K^+ ATPase produces some background stain in the gland cells, because staining was prolonged to detect any cells that might be weakly positive, but none of the IF-positive cells stained for H,K ATPase. In (**c, d**) from the mid-body of the stomach the same glands are labeled with both antibodies. No cells producing IF but not H^+,K^+ ATPase can be seen. Magnification (**a, b**) $\times 10$ objective, (**c, d**) $\times 20$

Fig. 3.

Double-label immunocytochemistry demonstrating cells expressing both intrinsic factor (*IF*) and pepsin. **a–c** An angularis biopsy from patient 3; **d–f** a mid-body biopsy from patient 6. **a, d** IF staining (*green*), **b, e** pepsin staining (*red*), **c, f** staining with both antibodies. **a–c** A cluster of cells from the angularis region, scored 2+ for ectopic IF, **d–f** a biopsy from the mid-body, scored 0 for clusters of cells producing ectopic IF. In (**a–c**) the lumen is in the upper right hand corner (the *arrowhead* identifies the apical surface), with the linear gastric glands extending toward the lower left hand corner (the *arrow* identifies the serosal surface). The clusters of cells are located mostly in the upper third of the crypt. **d–f** A region from the mid-body near the base of the crypts (the serosal surface is in the lower right hand corner). This region stains heavily for pepsin-positive cells (**e**). Linear crypts can be seen heading toward the lumen (off the picture) in the upper left hand corner. The *arrows* show 2 cells that were positive for both IF (**d**) and pepsin (**e**), confirmed by the double-labeled image in (**f**). The upper cell is shown in the *enlarged box*. Magnification (**a–c**) $\times 20$ objective, (**d–f**) $\times 40$

Fig. 4. Double-labeled immunofluorescence demonstrating cells expressing either intrinsic factor (*IF*; *green* stain) or the lectin Griffonia Simplicifolia II (*GSII*; *red* stain). **a–d** From the angularis of subject 18. **a, b** Staining with hematoxylin, **c, d** double-labeled immunofluorescence. The *red* staining of the expanded mucous neck cell region occurs in the mid-lower gland region. The *boxes* in (**a, c**) locate the GSII staining cells within the total gastric mucosa. The *right hand box* in (**a, c**) are magnified in (**b, d**). Magnification (**a, c**) $\times 20$ objective, (**b, d**) $\times 40$

Table 1

Characteristics of the nine patients studied for ectopic intrinsic factor in gastric mucosa

Case ^a	Gastric characteristics		Cobalamin absorption	
	Age (years)/sex	IF (nmol/l)	MAO (mEq/hr)	<i>H. pylori</i>
2	74/M	12.0	0	Negative
3	77/M	0.8	0	Negative
4	58/F	27.4	5.28	Positive
5	70/M	37.3	4.02	Positive
6	57/F	11.7	ND	Positive
7	66/F	ND	ND	Positive
12	44/F	25.0	8.64	Negative
16	72/M	44.5	13.91	Positive
18	48/M	33.1	2.56	Positive
Normal		>1.3	>5.0	

F Female, *FCM* food-cobalamin malabsorption, *IF* gastric intrinsic factor concentration, *M* male, *MAO* gastric maximal acid output, *ND* not determined

^a Each patient's number indicates the corresponding number used in the original report on gastric status in food-cobalamin malabsorption [21], from which the data in this table are taken

Table 2

Gastric histology and ectopic intrinsic factor (eIF) in the archived gastric biopsies of the nine patients with and without gastritis

Case ^d	Gastric corpus (midbody) ^b			Angularis		Gastric antrum (prepylorus) ^c			
	Gastritis ^d	Atrophy ^e	eIF ^f	Gastritis ^d	Atrophy ^e	eIF ^f	Gastritis ^d	Atrophy ^e	eIF ^f
2	3.0	4.0	1+	0	4.0	0	1.3	3.3	1+
3	1.5	4.0	0	2.0	0	1+	1.3	0	0
4	1.3	1.0	0	1.0	1.0	2+	3.0	1.0	1+
5	9.3	2.0	2+	11.0	3.0	1+	9.3	3.3	1+
6	5.3	2.0	1+	7.0	2.0	2+	4.5	3.3	1+
7	2.0	1.0	0	4.0	1.0	0	3.3	1.0	0
12	0.3	0.5	1+	0	0	0	0	0	0
16	5.3	1.0	1+	11.0	4.0	0	8.7	4.0	0
18	5.3	2.0	0	7.0	2.0	1+	7.3	0.3	1+
Scoring ranges	0–12.0	0–4.0	0–2+	0–12.0	0–4.0	0–2+	0–12.0	0–4.0	0–2+

^a Each subject's number in parentheses indicates the corresponding number in their original report [21]

^b Biopsy specimens taken from the greater curvature of the mid-body

^c Biopsy specimens taken from the prepyloric region

^d As detailed in an earlier report [21], the gastritis index (range 0–12) is the sum of the inflammatory cell findings (0–3 points for presence of neutrophils and 0–3 for mononuclear cells) and the epithelial cell findings (0–3 points for surface epithelium and 0–3 for pit epithelium). The mean score from 3 gastric sites sampled from each area is provided in each patient

^e As detailed in an earlier report [21], in the atrophy score: 0 reflects normal findings, 1–3 reflect progressive grades of superficial changes, and 4 reflects gastric atrophy. Mean scores from 2 sites are provided per gastric area

^f Ectopic intrinsic factor (eIF): presence of cell clusters (angularis and prepylorus) or single cells (mid-body) that are positive for IF but negative for H⁺, K⁺-ATPase, the immunochemical marker for the parietal cell