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Identification and characterization of rat intestinal trefoil factor: Tissue- and cell-specific member of the trefoil protein family

(intestine/goblet cell/growth factor)

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ABSTRACT **The trefoil peptide family encompasses a group of small proteins that appear to assume a distinctive secondary structure that leads to intrinsic resistance to protease digestion. Induction of these peptides has been associated with response to injury in the gastrointestinal tract and related organs. Using an oligonucleotide derived from N-terminal amino acid sequencing of a transformed growth-inhibiting protein, a cDNA was cloned from rat intestinal villus epithelial cells that encodes a protein 81 amino acids in length with the characteristic trefoil peptide cysteine residue motif. Northern blot analysis demonstrates specific expression of a single transcript of 0.43 kilobase in small and large intestinal epithelium in rat and man. Indirect immunofluorescent staining with antiserum raised using a synthetic peptide based on the predicted C-terminal sequence of this protein, designated intestinal trefoil factor, demonstrated that it is primarily expressed and secreted onto the intestinal surface by goblet cells, suggesting that it may be an important component of intrinsic mechanisms for defending mucosal integrity.**

Over the past several years, a wide variety of growth modulatory proteins has been found within the gastrointestinal tract. They encompass diverse peptides with demonstrated biological activities across many tissue types, such as the members of the epidermal growth factor and transforming growth factor β families (1–4). However, it is clear that additional peptides contribute to the regulation of proliferation and differentiation as well as defense of mucosal integrity. In the past few years, a number of small proteins expressed at various sites within the gastrointestinal tract and accessory organs that possess a broad range of biological activities that could contribute to mucosal defense have been identified. Designated trefoil peptides by Thim (5), who suggested that intrachain disulfide bonds may lead to three intrachain loops, this family includes the pS2 protein first identified as an estrogen-responsive gene product and subsequently found to be expressed normally in gastric mucosa as well as the porcine pancreatic spasmolytic polypeptide (PSP) produced in large amounts by the exocrine pancreas and its human counterpart, hSP (6–12). A homologous protein designated *Xenopus* spasmolysin (1xSL) has also been identified within frog skin (13, 14).

Direct study of the biological and biochemical properties of the trefoil proteins has been largely confined to PSP. This peptide has been found to possess a broad range of activities, including modulation of epithelial growth, intestinal motility, and acid secretion (15, 16). Although comparable evaluation of the effects of the other known trefoil peptides is lacking, circumstantial evidence suggests that they may possess properties contributing to mucosal response after ulceration. Induction of expression of hSP and pS2 has been reported in association with peptic ulcers and mucosal injury in inflammatory bowel disease (17, 18). These effects are especially intriguing in view of the demonstrated resistance of at least one trefoil peptide (PSP) to digestion by intraluminal proteases within the gastrointestinal tract (11). Thus, apparently undegraded PSP was recovered following prolonged exposure to the protease-rich environment of the gastrointestinal tract lumen. Moreover, PSP was found to exhibit its full range of biological activities following oral administration (15). These observations suggest that the trefoil family of peptides may be especially well suited to contribute to mucosal defense through a variety of mechanisms.

Earlier efforts in this laboratory to identify previously unrecognized proteins that contribute to the regulation of proliferation and commitment to differentiation among intestinal epithelial cell populations led to the isolation of at least two such proteins (19, 20). cDNA cloning has led to the identification of a member of the trefoil family that is specifically expressed in the goblet cell population within epithelium of the large and small intestine. This factor may be an important component of host defenses that serve to maintain the integrity of the intestinal mucosa and facilitate recovery after injury.†

MATERIALS AND METHODS

Molecular Cloning of Rat Intestinal Trefoil Factor (ITF). Rat intestinal epithelial cells were isolated by the method of Weiser (21), and mRNA was prepared by oligo(dT)-cellulose chromatography after isolation of total RNA by the guanidine isothiocyanate/CsCl cushion technique using epithelial cell fractions 3 and 4. cDNA was prepared by the method of Gubler and Hoffman (22) after methyl mercury treatment of 10 μg of poly(A)$^+$ RNA; cDNA was ligated into the *Eco*RI-digested λZAP II vector (Stratagene) to yield a library with an estimated 10^6 recombinants. This library was screened with an oligonucleotide probe containing deoxyinosine at degenerate positions complementary to the predicted nucleotide sequence dictated by N-terminal sequencing of a malignant effusion-related transformed cell growth-inhibiting factor (19). The probe was end-labeled using T4 polynucleotide kinase and [γ-^{32}P]ATP. Filters were prehybridized at 42°C for 2 hr in 5× SSC (1× SSC = 0.15 M NaCl/15 mM sodium citrate)/0.1% SDS and 5× Denhardt's solution in the presence of 100 μg of salmon sperm DNA per ml. The labeled probe was added at 5×10^5 cpm/ml and hybridization was carried out at 42°C for 15 hr. Filters were then washed twice

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Abbreviations: PSP, porcine pancreatic spasmolytic polypeptide; ITF, intestinal trefoil factor; hSP, human spasmolytic polypeptide; xSL, *Xenopus* spasmolysin.

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M80826).

in 5× SSC/0.1% SDS at room temperature and finally washed in 5× SSC/0.1% SDS at 50°C for 2 min. Five independent but cross-hybridizing recombinants were obtained and plaque purified. cDNA sequences were determined using the dideoxyribonucleotide chain-termination reaction with dATP[^{35}S] and Sequenase (United States Biochemical). Reaction products were resolved on denaturing polyacrylamide gels by standard techniques (23). Sequence comparisons were made using the ALIGN program and the program of Devereux *et al.* (24) in conjunction with GenBank (Release 68.14) and EMBL (Release 26.0) data bases.

RNase Protection Analysis. For determination of the 5′ end of rat ITF cDNA, poly(A)$^+$ mRNA from isolated rat intestinal epithelial cells was hybridized to the end-labeled oligonucleotide 5′-ATACATTGG CTTGGAGAT AGGCCAACA AATTCCTGG GC-3′ complementary to nucleotides 81–118 of the rat ITF cDNA according to established methods at 42°C for 18 hr in 40 mM Pipes, pH 6.4/1 mM EDTA, pH 8.0/0.4 M NaCl/80% formamide (25). Reverse transcription was carried out at 37°C for 2 hr and RNase digestion was performed at 37°C for 30 min. Radiolabeled RNA·DNA hybrids were analyzed by electrophoresis on 8% polyacrylamide/7 M urea gels.

Northern Blot Analysis. mRNA was prepared from various rat tissues and isolated intestinal epithelial cells as described above, denatured with formamide, fractionated by electrophoresis on 1.0% formaldehyde/agarose gels, and transferred to nylon membrane as detailed earlier (25). mRNA was also prepared from the IEC-6 cell line derived from the normal rat intestinal crypt epithelium (26). The *Eco*RI insert of the ITF cDNA clone was radiolabeled by nick-translation, prehybridized for 2 hr in 30% formamide/5× SSC/0.2% SDS and 5× Denhardt's solution in the presence of 500 μg of salmon sperm DNA per ml, and hybridized at 60°C for 15 hr in 30% formamide/5× SSC, 5× Denhardt's solution, 10% dextran sulfate, and 100 ng of salmon sperm DNA per ml. Filters were washed once at room temperature and washed twice at 67°C in 2× SSC/0.1% SDS for 15 min prior to autoradiography for 16 hr at −80°C. Approximately 3 μg of poly(A)$^+$ RNA was loaded per lane and verified through rehybridization with a probe for the constitutively expressed transcript for glyceraldehyde-3-phosphate dehydrogenase (27).

Preparation of Anti-ITF Antiserum and Immunofluorescent Staining. A conventional antiserum was produced by immunization of New Zealand White rabbits with the 21-residue peptide DSSIPNVPWCFKPLQETECTF reflecting the deduced C-terminal sequence of ITF. After initial immunization with 100 μg in 1.0 ml of complete Freund's adjuvant by intradermal injection, the rabbits were given booster immunizations at 4-week intervals with 100 μg of peptide in incomplete Freund's adjuvant. Serum was prepared from blood obtained 10 days after booster immunization.

Frozen sections (≈3 μm) of rat small intestine and other tissues were prepared after embedding in OCT compound (TissueTek, Miles) and incubated with the specific anti-ITF antiserum or preimmune serum [diluted 1:100 in phosphate-buffered saline (PBS)] for 30 min. In addition, some sections were incubated with the specific antiserum in the presence of an excess (25 μg/25 μl) of the synthetic peptide used as immunogen. Sections were then washed three times with excess PBS and stained by addition of fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin diluted 1:25. After 30 min, excess reagent was aspirated, sections were washed again as before, and fluorescent staining was evaluated.

RESULTS

Earlier work in this laboratory demonstrated a peptide present within human malignant effusions, provisionally designated TGIF, that could inhibit the anchorage-independent growth of several tumor-derived cell lines (19). This factor was purified and the N-terminal amino acid sequence was identified by microsequencing. A degenerate oligonucleotide based on this peptide sequence was used to screen a number of tissues and cell lines by Northern blot analysis. These pilot studies demonstrated the presence of a transcript 0.4–0.5 kilobase (kb) in rat intestinal epithelial cells that hybridized to the mixed oligonucleotide. Therefore, to characterize the structural features of this protein and its relationship to previously described growth-modulating peptides, a cDNA library was prepared from mRNA obtained from isolated primary rat enterocytes and screened with the degenerate oligonucleotide probe. Plaques hybridizing with the probe in the initial screening were purified through three subsequent rounds of plating and hybridization. These procedures resulted in the isolation of a pure recombinant containing a 0.43-kb insert. As demonstrated in Fig. 1, the 0.43-kb cDNA encompassed an open reading frame of 81 amino acids. The 5′ end of this cDNA was confirmed by primer extension/RNase protection analysis using mRNA from rat intestinal epithelial cells (Fig. 2). The latter study confirms the impression derived from the recovery of the same 5′ end from five separately isolated additional clones that the sequence indeed reflects a complete cDNA.

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5'-gaagtttgcgtgctgccatggagaccagagccttctggacaaccctgctgctggtcctgg
                          M  E  T  R  A  F  W  I  T  L  L  L  V  L  V
                                   ▼
   ttgctgggtcctcctgcaaagcccaggaatttgttggcctatctccaagccaatgtatgg
     A  G  S  S  C  K  A  Q  E  F  V  G  L  S  P  S  Q  C  M  A

   ctccaacaaatgtcagggtggactgtaactaccccactgtcacatcagagcagtgtaaca
     P  T  N  V  R  V  D  C  N  Y  P  T  V  T  S  E  Q  C  N  N

   accgtggttgctgttttgactccagcatcccaaatgtgccctggtgcttcaaacctctgc
     R  G  C  C  F  D  S  S  I  P  N  V  P  W  C  F  K  P  L  Q

   aagagacagaatgtacattttgaagctgtccaggctccaggaagggagctccacaccctg
     E  T  E  C  T  F

   gactcttgctgatggtagtggcccagggtaacactcacccctgatctgctccctcgcgcc

   ggccaatataggagctgggagtccagaagaataaagaccttacagtcagcacaaggctgt

   tctaattgcgg-3'
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FIG. 1. Molecular cloning of rat ITF. An oligo(dT)-primed cDNA library was prepared from mRNA isolated from rat intestinal villus epithelial cells [fractions 3 and 4 obtained by the method of Weiser (21)]. The library was screened with an oligonucleotide probe containing deoxyinosine at degenerate positions complementary to the predicted nucleotide sequence dictated by N-terminal sequencing of a malignant effusion-related transformed cell growth-inhibiting factor (19). Sequences of five separate cDNA clones were determined by the dideoxyribonucleotide chain-termination reaction with dATP[^{35}S] after four rounds of plaque purification by standard techniques (23, 25). The arrowhead indicates the predicted site of cleavage of propeptide to yield a mature processed peptide (see Fig. 3).

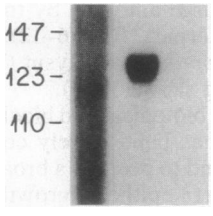

FIG. 2. Determination of the 5′ end of rat ITF cDNA. Poly(A)$^+$ mRNA from isolated rat intestinal epithelial cells was hybridized to the end-labeled oligonucleotide 5′-ATACATTGG CTTGGAGAT AGGCCAACA AATTCCTGG GC-3′ complementary to nucleotides 81–118 of the rat ITF cDNA according to established methods (25) under conditions detailed in the text. Radiolabeled RNA·DNA hybrids were analyzed by electrophoresis on 8% polyacrylamide/7 M urea gels. Left lane, molecular weight standards (indicated in $M_r \times 10^{-3}$); right lane, protected hybrid.

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ITF  KAQEFVGLSPSCGMNPTNV-RVDCNYPTVTSECNNRGCCFDSSIPNVPWCFK-----PLQ-----ETECT-----F
pS2  EAQ------TETCIVAPRE-RQNCGFPGVTPSQCANKGCCFDDTVRGVPWCFY-----PNTIDVPPEEECE-----F
PSP  -----EKPAACRCSRQDPKNRVNCGFPGITSDQCFTSGCCFDSQVPGVPWCFK-----PLP-----AQESEECVMEV
xSL  ----------QDCSVAPNM-RVNCGYPTVTEADCRAVGCCFDSSILNTKWCFYNATAGPIK-----KLECS-----G
HSP  ------KPSPCQCSRLSPHNRTNCGFPGITSDQCFDNGCCFDSSVTGVPWCFH-----PLP-----KQESDQCVMEV
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FIG. 3. Comparison of ITF predicted amino acid sequence (omitting presumptive signal sequence) with known members of the trefoil family, including pS2, PSP, HSP (hSP), and xSL. Cysteine residues comprising the trefoil motif are presented in bold type. Comparisons were made using the ALIGN program.

Comparison of the amino acid sequence encoded by the cDNA presented in Fig. 1 with available data bases demonstrates close homology with the known members of the trefoil peptide family, including absolute conservation of the cysteine residues that are presumed to participate in the trefoil motif and contribute to their protease resistance (Fig. 3). These include PSP and its human homology hSP as well as the human pS2 protein and xSL. It should be noted that PSP and hSP are approximately twice as large as the intestinal protein. The latter has a single so-called "P" domain (the trefoil motif) in contrast to the two P domains in the former. In having a single P domain, the intestinal protein is similar to the pS2 and xSL members of the trefoil protein family. However, the extent of homology of the intestine-derived protein was essentially equivalent for each of the P domains of PSP and hSP (the second or carboxyl P repeat is indicated in Fig. 3). By analogy to the pS2, hSP, and PSP proteins, this protein designated rat ITF, appears to include a 21-residue signal sequence with a predicted length of 60 amino acids in the mature ITF. In addition to the preservation of the consensus cysteine residues, significant preservation of homology is also seen in the residues adjacent to the sites of presumed intrachain disulfide bond formation. The greatest divergence is observed at the N and C termini outside the region involved in putative intrachain loop formation. The secondary structure of ITF suggested by the homology with other members of the trefoil peptide family as postulated by Thim (5) is presented in Fig. 4 and includes the three characteristic intrachain loops formed of 13, 16, and 18 amino acids.

The tissue distribution of expression of rat ITF was evaluated at the level of mRNA by Northern blot analysis. Using the full-length cDNA insert as probe, this analysis reveals marked specificity. As demonstrated in Fig. 5, a single transcript of 0.43 kb was abundant in rat small and large intestine but was not found in the remainder of the gastrointestinal tract or accessory organs. Specifically, rat ITF was not found in the stomach or pancreas, which earlier studies have shown to be the site of production of the pS2 and hSP proteins in humans and their homologues in other animals. Thus ITF exhibits a pattern of tissue-specific expression within the gastrointestinal tract that is complementary to that of previously identified members of this protein family. ITF was not detected in the liver, pancreas, and stomach. ITF was present in all regions of the small intestine but appeared to be most abundant in the distal small intestine. ITF appeared to be expressed by differentiated epithelial populations isolated from intestinal mucosa; it was not found in the rat intestinal crypt epithelial-derived cell line IEC-6 (26). Indeed, ITF was not expressed in any non-gastrointestinal-associated tissue with the single exception of the rat kidney. The transcript present in the kidney appears to be identical in its sequence with that expressed in the large and small intestine (K.L.-D. and D.K.P., unpublished). A transcript hybridizing to the rat ITF in Northern blot analysis was found to be present in human small and large intestinal tissue as well. Efforts are necessary to clone this human homologue.

To further define the pattern of expression of ITF, a conventional antiserum was prepared by immunization with a synthetic peptide reflecting the predicted C-terminal sequence of ITF. This sequence was chosen to preclude cross-reactivity with other known trefoil factors. As demonstrated in Fig. 6, the ITF protein was specifically found within goblet cells, where staining of the theca is apparent, and in the viscoelastic mucosa coat overlying the mucosal surface. Staining could be completely blocked by the addition of the soluble peptide used for immunization. However, specific staining could not be blocked by preincubation with a synthetic peptide from the C-terminal 20 amino acids of PSP.

No staining was observed when tissue sections were incubated with preimmune serum. No specific staining was observed when rat gastric mucosa or pancreatic tissue was incubated with the anti-ITF antiserum. Finally, it should be noted that the antiserum prepared against the deduced rat protein also specifically stained goblet cells within human

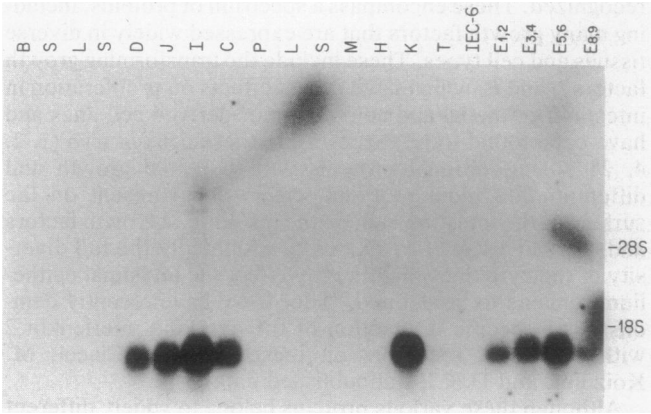

FIG. 5. Tissue specificity of ITF expression. mRNA was prepared from various rat tissues and isolated intestinal epithelial cells, and Northern blot analysis was carried out using a complete cDNA probe for ITF obtained as the *Eco*RI insert radiolabeled by nick-translation. Approximately 3 μg of poly(A)$^+$ RNA was loaded per lane and verified through rehybridization with a probe for the constitutively expressed transcript for glyceraldehyde-3-phosphate dehydrogenase (not shown). B, brain; S, salivary gland; L, lung; S, stomach; D, duodenum; J, jejunum; I, ileum; C, colon; P, pancreas; L, liver; S, spleen; M, skeletal muscle; H, heart; K, kidney; T, testes; IEC-6 cells (26). E1,2, E3,4, E5,6, and E8,9 represent isolated primary rat intestinal epithelial cells ranging from villus tip cells (E1,2) to populations enriched in undifferentiated mitotically active crypt cells (E8,9) as described (21).

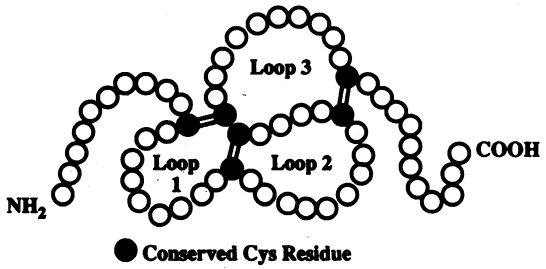

FIG. 4. ITF: Predicted secondary structure [based on model of Thim (5)].

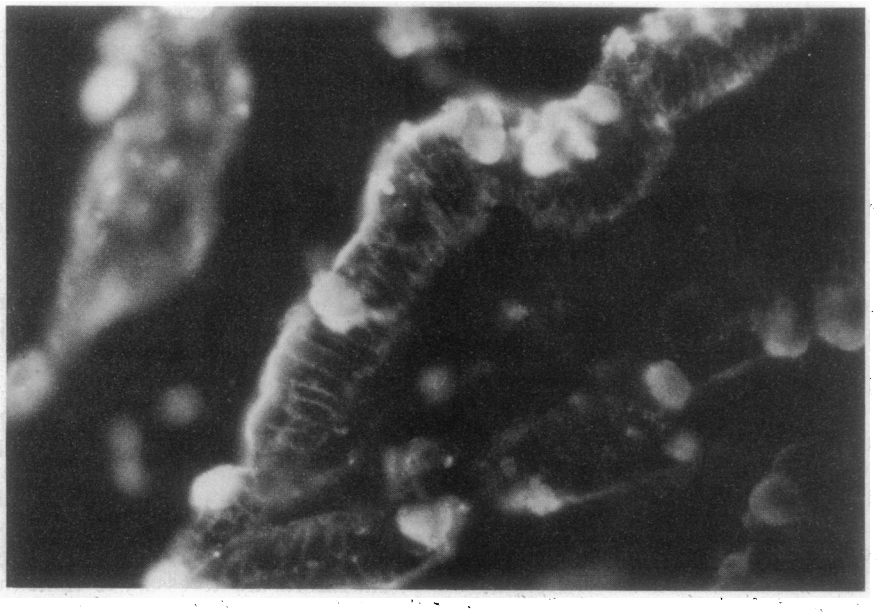

FIG. 6. Cellular distribution of ITF in the small intestine. A conventional antiserum was produced by immunization with the 21-residue peptide reflecting the predicted C-terminal sequence of ITF. A frozen section of rat small intestine tissue was incubated with the specific anti-ITF antiserum (1:100), washed three times, and stained by addition of fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin. (×400.)

colonic and human small intestinal mucosa but not gastric or pancreatic tissue (B. Sands and D.K.P., unpublished). These findings suggest that ITF is predominantly or exclusively expressed by goblet cells and secreted onto the luminal surface where it may contribute to defense of mucosal integrity. The implied specific expression by goblet cells is consistent with the absence of the ITF transcript in the IEC-6 cell line.

DISCUSSION

Over the past several years a number of proteins that may contribute to regulation of intestinal epithelial cell growth and preservation of the integrity of the mucosal surface have been recognized. These encompass a spectrum of proteins, including many growth factors that are expressed widely in diverse tissues and cell types. These include the transforming growth factors α and β, which have potent effects on proliferation in intestinal epithelial and colonic tumor-derived cell lines and have been found to be expressed in these cells *in vivo* (1, 2, 4, 28–30). Additional proteins with potential growth and differentiation modulatory effects in cells present on the surface of the intestine include the insulin-like growth factors and a broad array of cytokines. Undoubtedly the full diversity of the cytokines that directly affect the intestinal epithelium remains to be defined. Indeed, we have recently demonstrated specific interaction of the cytokine interleukin 2 with functional receptors on these cells (C. Ciacci, M. Koizumi, and D.K.P., unpublished data).

Although these various proteins belong to widely different structural families, they share some common broad similarities in the mechanisms through which they influence intestinal epithelial populations. These factors are produced by cells within the lamina propria or by the intestinal epithelial cells themselves and mediate their activities through interactions with specific receptors at the basolateral lamina surfaces. Although it has been suggested that some peptides, notably epidermal growth factor, might be secreted into the gastrointestinal tract lumen and act from this site, intraluminal or mucosal surface proteases, including those produced by the pancreas and intestinal mucosa itself, as well as bacterial enzymes have been found to lead to rapid degradation (31). The present studies identify a further protein, ITF, expressed by the small and large intestinal mucosa that could represent a categorically different class of regulatory protein capable of acting at the luminal surface. This conclusion is suggested by immunolocalization of ITF to the goblet cell population and the presence of the protein in the viscoelastic coat overlying the mucosal surface. Indeed, these observations suggest that the overlying mucin glycoprotein-containing matrix present on the mucosal surface may act as a depot for bioactive regulatory proteins.

A number of observations support the impression that antiserum prepared against the C terminus specifically identified ITF in intestinal mucosa. These include the ability to block binding by the immunizing peptide but not synthetic peptide of other trefoil factors. In this context, it should be noted that characterization of hSP has shown a significantly higher degree of amino acid sequence among diverse mammalian species relative to the other human trefoil protein pS2. Thus, hSP, PSP, and their mouse homolog differ in only 2 amino acids among the C-terminal 21 residues; in contrast, each of these proteins differs in >8 amino acid residues when compared to this region of ITF that was used to produce the anti-ITF antiserum. More importantly, the pattern of tissue-specific expression was complementary rather than consistent with the distribution of the PSP/hSP and pS2 proteins in other species. Thus, the antiserum stained only colonic and intestinal mucosa in rat as well as man, in contrast to the gastric and pancreatic localization of other trefoil factors. The latter observations further suggest the presence of a protein in human colonic and intestinal mucosa highly homologous to the rat ITF as suggested by the results of Northern blot analysis. Efforts are necessary to characterize the human protein; preliminary results of cDNA cloning indicate considerable homology with the rat protein.

The secondary and tertiary structural features of ITF implicit in its primary amino acid sequence suggest that this factor is resistant to proteolytic degradation and therefore capable of retaining biological activity in the environment of the gastrointestinal tract lumen. PSP, a homolog of ITF expressed in the pancreas and stomach, has been shown directly to exert biological activity when given by an oral route to animals (11, 15). Importantly, characterization of the PSP reisolated after intraluminal administration, demonstrated the absence of protease degradation. Resistance to

protease digestion despite the presence of multiple potential sites for cleavage by proteases commonly present in the gastrointestinal tract is presumed to derive from extensive intrachain disulfide bond formation that may contribute to the putative three-loop motif that distinguishes this family of proteins. Insofar as ITF demonstrates absolute conservation of the trefoil consensus cysteine residues, it is anticipated that this member of the trefoil family intrinsic to the intestinal mucosa is similarly resistant to degradation, facilitating biological activity after secretion onto the luminal surface.

The presence of ITF as a secretory protein destined for the luminal surface suggests that the protease resistance thought to derive from the trefoil motif is inherently linked to its physiologic activities. The full range of these biological activities remains to be defined. Studies on PSP have demonstrated effects on acid secretion and motility as well as epithelial growth (15, 16). Identification of ITF follow screening of a cDNA library based on the microsequencing of transformed growth inhibiting factor, a potent growth-modulating protein present in small concentration in human malignant effusions. It is of interest to note that another member of the trefoil family, pS2, was initially recognized due to its frequent "ectopic" expression by estrogen-responsive breast carcinomas (6). Further characterization proved that this protein was normally expressed exclusively within the gastric mucosa (8).

A recent series of observations suggest that trefoil proteins may play an especially important role as growth factors that contribute to healing after mucosal injury throughout the gastrointestinal tract (17, 18). Most notably, the trefoil proteins pS2 and hSP have been found in humans in association with peptic ulcers within the stomach as well as sites of small intestinal ulceration due to Crohn disease. Enhanced expression of the trefoil protein hSP has also been noted in association with pancreatic ductular injury resulting from pancreatitis. Wright *et al.* (32) have speculated that these factors are produced by a distinctive growth factor-producing cell. The identification of ITF suggests that it may be the trefoil factor intrinsic to the small and large intestine that serves to promote mucosal integrity at this site. It will be of interest to examine the relative level of expression of this factor in conditions resulting in mucosal injury—e.g., inflammatory bowel disease.

Little is known about the mechanism of action of the trefoil factors. Initial studies with PSP indicate that specific surface receptors that are coupled to adenyl cyclase are present on epithelial cells (33). Purification of the ITF protein through immunoaffinity chromatography and production of the protein through expression cloning techniques should facilitate characterization of the spectrum of its biological activities and identification of specific ITF receptors.

Collectively, these observations demonstrate the presence of a class of proteins abundantly expressed throughout the gastrointestinal tract that may be hypothesized to play an essential role in the maintenance of mucosal integrity and normal gastrointestinal tract function. The functional importance of these proteins is suggested by their apparent structural adaptation to resist degradation in the luminal environment and induction of enhanced expression in association with injury to the mucosal barrier. Characterization of the biological activities of the ITF will clarify the spectrum of potential effects it may have on physiologic intestinal functions. Conversely, further study of the structure of this protein may facilitate modification of other peptides to achieve stability within the gastrointestinal tract and preservation of biological activities after luminal or oral administration.

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