Regulation of Sucrase-Isomaltase Gene Expression in Human Intestinal Epithelial Cells by Inflammatory Cytokines*

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Using metabolic labeling techniques in human intestinal epithelial cell lines in tissue culture and in situ hybridization techniques in normal and inflamed (Crohn's) intestine, recent studies have shown that there is synthesis of acute phase proteins in enterocytes. Moreover, these studies have shown that acute phase protein biosynthesis in enterocytes is regulated by inflammatory cytokines in a manner characteristic of the physiologic acute phase response. In the course of these studies it was noticed that one inflammatory cytokine, interleukin-6 (IL-6), mediated selective down-regulation of the enterocyte-specific, differentiation-dependent integral membrane protein sucrase-isomaltase (SI) in the Caco2 intestinal epithelial cell line. In the current study we examined the effect of several other inflammatory cytokines interleukin-1 (IL-1α), tumor necrosis factor α (TNFα), and interferon γ (IFNγ) on synthesis of SI in Caco2 cells, examined the possibility that inflammatory cytokines affect the synthesis of other enterocyte integral membrane proteins using lactase as a prototype, and examined the possibility that SI gene expression was down-regulated in villous enterocytes in vivo during the local inflammatory response of Crohn's disease. The results show that IL-6 and IFNγ each mediate a decrease and TNFα mediates an increase in synthesis of SI in Caco2 cells. The magnitude of down-regulation by IL-6 and IFNγ is significantly greater than the up-regulation by TNFα. IL-1β has no effect on synthesis of SI. Synthesis of lactase is not affected by any of the cytokines. There is a marked specific decrease in SI gene expression in villous enterocytes in acutely inflamed Crohn's ileum as compared to adjacent uninflamed ileum and normal ileum. Taken together, these data show that inflammatory cytokines have specific and selective effects on the expression of the brush border hydrolase SI in tissue culture and in vivo and provide evidence for a previously unrecognized mechanism for disaccharidase deficiency in intestinal inflammation.

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The host response to injury, the acute phase response, is characterized by a coordinate series of physiological changes, including marked changes in plasma concentrations of secretory glycoproteins termed “acute phase reactants” (reviewed in Refs. 1–3). In recent years several extracellular soluble factors thought to be generated at the site of injury and able to circulate via the bloodstream to remote sites have been identified and implicated in coordination of the acute phase response. The most well characterized of these cytokines include IL-6, IL-1α, IL-1β, TNF-α, and IFN-γ. These cytokines have been shown to be capable of mediating in tissue culture and in vivo many of the changes in hepatic gene expression that characterize the acute phase response.

Although the acute phase reactants are predominantly derived from liver, there is now evidence that acute phase reactants are produced in extrahepatic tissues and cell types (4–9). Moreover, there is evidence that expression of these gene products in extrahepatic sites is regulated by cytokines in a manner characteristic of the physiologic acute phase response. For instance, we have found that positive acute phase reactant α1-antitrypsin (α1-AT) is expressed in human intestinal epithelial cell lines Caco2 and T84 (10) and, moreover, that IL-6 mediates an increase in synthesis of α1-AT in these cell lines (11). Caco2 and T84 cells were found to synthesize many other positive and negative acute phase reactants including α1-antichymotrypsin, serum amyloid A (SAA), fibrinogen, ceruloplasmin, complement proteins C3, C4, and factor B, α-fetoprotein, and transferrin (12). Expression of all these acute phase reactants was regulated by cytokines IL-6 and IL-1α in exactly the same way that they are regulated in liver cells. In fact, effects of IL-1 and IL-6, which were found to be additive, synergistic, and antagonistic in liver cells in previous studies, were also observed in the intestinal epithelial cell lines. Finally, for at least one of these proteins, α1-AT, in situ hybridization analysis of normal and inflamed human intestine has shown that the expression and regulation of the acute phase gene in the cell lines accurately recapitulates expression and regulation of this gene in vivo.

During the course of these studies of the acute phase response in the Caco2 cell line (12), we found that IL-6 had a marked and selective down-regulating effect on the expression of sucrase-isomaltase (SI). SI is not an acute phase reactant, but it is an enterocyte-specific, differentiation-dependent integral membrane protein. It is expressed in the adult small intestine where, as a brush border hydrolase, it is involved in the final steps of digestion of sucrose, starch and glycogen. Its expression is limited to the villous enterocyte (14–16). In fact, together with lactase and intestinal alkaline phosphatase it is often used as a marker of terminal differentiation of enterocytes. SI is synthesized as a co-translationally glycosylated high mannose precursor with a molecular mass of 210 kDa (17). This precursor is also subject to O-glycosylation in the Golgi and is finally targeted to the apical membrane. After SI
Regulation of Sucrase-Isomaltase by Inflammatory Cytokines

has reached the apical membrane as a single-chain glycoprotein, it undergoes post-insertional cleavage by intraluminal pancreatic trypsin into the sucrase (molecular mass ~ 130 kDa) and isomaltase (molecular mass ~ 145 kDa) subunits (18, 19).

In this study we examined the possibility that inflammatory cytokines other than IL-6 mediate changes in expression of the SI gene in the model enterocyte cell line Caco2, whether inflammatory cytokines regulate expression of other brush border hydrolases in Caco2 cells, and whether SI gene expression is regulated in a local site of inflammation, acutely inflamed Crohn’s ileum.

Experimental Procedures

Materials—Ribonuclease A was purchased from Sigma, ribonuclease T1 was purchased from Life Technologies, Inc., and DNase I and ribonuclease inhibitor were purchased from Promega Biotech (Madison, WI). [35S]-UTP and [32P]-CTP were purchased from DuPont NEN. Baculovirus-derived human IL-6 was kindly provided by Drs. L. May and P. B. Sehgal (New York, NY). Escherichia coli-derived human IL-6, IL-1β, and TNF-α were provided by Dr. J. McKearn (St. Louis, MO). Recombinant human IL-6, IL-1β, TNF-α, and IFN-γ were also purchased from R & D Systems (Minneapolis, MN). A murine monoclonal antibody to human SI, HSI 14 (20), was kindly provided by Dr. A. Quaroni (Ithaca, NY). Twelve different antibodies to lactase were used: a polyclonal rabbit anti-human lactase antibody purchased from R. Dr. Liow (London, United Kingdom). Rabbit anti-human α-AT was purchased from Dako (Santa Barbara, CA), and goat anti-human complement C3, C4, and factor B antibodies purchased from Atlantic Antibodies (Scarborough, ME).

Cell Culture Biosynthetic Labeling, Immunoprecipitation, and SDS-PAGE—Caco2 cells (22, 23) were maintained in culture and subcultured for experiments exactly as described in our previous studies (10, 12). A subclone of Caco2 cells, Caco2/15, which has undergone only a limited number of passages and has higher levels of sucrase-isomaltase expression (24), was kindly provided by A. Quaroni (Ithaca, NY). Twelve days after reaching confluence, monolayers of these cells in six-well plates were rinsed well and then incubated at 37°C for time intervals up to 24 h in serum-free control medium with or without cytokine(s). Cells were then rinsed again and incubated at 37°C for 30–45 min in the presence of methionine-free medium containing 250 µCi/ml [35S]methionine. Cells were then solubilized and cell lysates subjected to clarification and trichloroacetic acid precipitation to determine relative total protein synthesis according to previously described protocols (10). Aliquots of cell lysate were incubated overnight at 4°C in 1% Triton X-100, 1.0% SDS, 0.5% deoxycholic acid, with excess anti-body. Immune complexes were then precipitated with excess formalin-fixed, stabilized secondary antibody protein A (immunoprecipitin, Life Technologies Inc.), washed, released, and measured on a LKB laser densitometer from LKB Instruments, Inc. (Houston, TX) was used for scanning of fluorograms.

Immunostaining—equal and ideal tissue was obtained from 2 different organ donors through the Mid America Transplant Association (St. Louis, MO). These studies were approved by the Human Studies Committee of Washington University School of Medicine. Ideal tissue from 2 patients with Crohn’s disease was obtained at the time of ileocolonic resection. Sections of ileum with acute inflammation were compared to adjacent uninfamed sections from each patient. Adjacent sections were fixed in formalin, stained with hematoxylin and eosin, and examined for routine histology in the Department of Anatomic Pathology. Acute inflammation was defined by conventional pathologic criteria. For immunostaining, cryostat sections (8 µm thick) of jejunum and ileum were fixed in 4% paraformaldehyde and prehybridized by proteinase K digestion (10 µg/ml for 30 min). Hybridization with the [35S]-UTP-labeled sense probe for 5′ to 6′ at 4°C for 24 h in 60% formamide, 15% dextran sulfate, 1× Denhardt’s solution, 1× EDTA, 10× Tris-Cl (pH 8), and 400 µm NaCl. Sections were then subjected to RNase A digestion to remove all nonhybridized single-stranded RNA, washed at stringencies of up to 0.1× SSC at 55°C for 30 min, and exposed to x-ray film at 4°C. The slides were developed 21 days after the emulsion dipping. Cellular expression of mRNA was indicated by the presence of silver grains. "Background" signal was defined as the density of grains on the glass slide, where no tissue was present. To determine which the hybridization signal was specific for probe, sequential sections of the same tissue were hybridized under identical conditions using [35S]-UTP-labeled sense probe. Using the sense probe, scattered refractive bodies were found over tissue and slide, but no specific hybridization signal was ever found.

RESULTS

Effects of IL-6, IL-1β, IFN-γ, and TNF-α on Biosynthesis of SI in Caco2 Cells—In previous studies we found that IL-6 mediated a marked decrease in synthesis of SI in Caco2 cells (12). Here we compared the effect of IL-6 to that of several other cytokines (Fig. 1). Caco2 cell monolayers were incubated at 37°C for 24 h with control serum-free medium or medium supplemented with IL-6 alone, IL-1β alone, IL-6 and IL-1β

![Fig. 1. Effect of inflammatory cytokines on synthesis of SI, factor B (Bf), C3, and C4 in Caco2 cells.](image)
Regulation of Sucrase-Isomaltase by Inflammatory Cytokines

Fig. 2. Effect of several different concentrations of IL-6 and IFNγ on synthesis of SI (top panels) and C3 (bottom panels) in Caco2 cells. The protocol was identical to the protocol described in the legend for Fig. 1. Lane 1, control serum-free medium; lane 2, 10 ng/ml IL-6; lane 3, 50 ng/ml IL-6; lane 4, 100 ng/ml IL-6; lane 5, 200 units/ml IFNγ; lane 6, 1000 units/ml IFNγ; lane 7, 5000 units/ml IFNγ. In this particular experiment, there was a greater decrease in SI after treatment with 200 units/ml IFNγ than after 100 units/ml or 5000 units/ml IFNγ. This is probably due to loading because there was a lesser decrease in SI after treatment with 200 units/ml IFNγ than after 1000 or 5000 units/ml in three other experiments (data not shown).

Together, or IFNγ. Monolayers were rinsed extensively and then pulse-radiolabeled with [35S]methionine for 30 min. Monolayers were then lysed, subjected to Immunoprecipitation with anti-SI antibody, and immunoprecipitates analyzed by SDS-PAGE/fluorography. A single radiolabeled polypeptide close to the 200-kDa marker was identified in control Caco2 cells in this experiment and in all subsequent experiments. Because this –200-kDa radiolabeled polypeptide was identified after a relatively short pulse interval, it was thought to correspond to pro-SI. There is a marked decrease in pro-SI in Caco2 cells incubated with IL-6 alone or IFNγ alone. This decrease is also seen in Caco2 cells incubated with both IL-1β and IL-6 but not with IL-1β alone, indicating that IL-6 is responsible for the down-regulation seen with combination of the two cytokines. The effects are highly selective and specific, as shown by sequential Immunoprecipitation of the same cell lysates with antibodies to positive acute phase complement proteins factor B, C3 and C4 (Fig. 1, second, third, and fourth panels). There is an increase in synthesis of factor B mediated by IL-6, IL-1β, and IFNγ and a synergistic increase in synthesis of factor B mediated by the combination of IL-1β and IL-6. In the case of C3, there is an increase in synthesis mediated by IL-1β alone or when together with IL-6, IL-6 alone and IFNγ alone mediated minimal increases in synthesis of C3. Synthesis of C4 is increased in response to IFNγ but is not affected by IL-6 or IL-1β. All of these changes in synthesis of acute phase complement proteins are consistent with changes seen previously in Caco2 cells (12), as well as in HepG2 cells (3), and are characteristic of changes in plasma concentration of these proteins during the inflammatory response in vivo (3).

Next we examined the possibility that regulation of SI biosynthesis by inflammatory cytokines was concentration-dependent. In Fig. 2, the results show that IL-6 and IFN-γ, indeed, mediate concentration-dependent decreases in SI biosynthesis (Fig. 2, upper panels). An effect was evident at concentrations of IL-6 as low as 10 ng/ml and of IFNγ as low as 200 units/ml. These concentration-dependent effects were specific as shown by sequential Immunoprecipitation for C3. There is a concentration-dependent increase in C3 mediated by IFNγ (Fig. 2, lower panels). IL-6 has minimal effects on synthesis of C3.

We also examined the effect of TNFα on synthesis of SI (Fig. 3, left panel). In contrast to IL-6 and IFNγ, TNFα mediated a modest concentration-dependent increase in SI synthesis in Caco2 cells. The effect was evident at concentrations of TNFα as low as 10 ng/ml. The magnitude of this increase was less than that mediated by TNFα on synthesis of C3 (Fig. 3, right panel) and less than the magnitude of the decrease in SI biosynthesis mediated by IL-6 and IFNγ. Quantitative analysis by densitometric scanning of five separate experiments shows that the optimal effect of IL-6 on SI biosynthesis resulted in a decrease to 37.4 ± 19.0% of control, of IFNγ resulted in a decrease to 51.3 ± 22.1% of control, and of TNFα resulted in an increase to 170.6 ± 21.0% of control.

The effects of IL-6, IL-1β, IFNγ, and TNFα on SI could not be attributed to general effects on cell number or metabolic activity because there was no significant effect on DNA content or on total trichloroacetic acid-precipitable protein determinations in these experiments (data not shown). Moreover, changes in cell number or metabolic activity would not explain the increase in synthesis of some proteins, decrease in others, and no change in still others on sequential Immunoprecipitation. Finally, examination of a subclone of Caco2 cells expressing high levels of SI under basal conditions, Caco2/15 cells, gave identical results (data not shown).

Next we examined the effect of cytokines on synthesis of another enterocyte-specific integral membrane disaccharidase, lactase (Fig. 4). In the same experiments in which selective and specific changes in synthesis of SI and complement proteins are noted, there was no significant change in the synthesis of lactase. There was also no change in the synthesis of another enterocyte integral membrane protein, alkaline phosphatase (data not shown). At least with respect to inflammatory cytokines in this model system, there appears to be discordant regulation of the two disaccharidases and the three enterocyte membrane proteins. The results of the experiments with lactase and alkaline phosphatase, which are considered markers of terminal differentiation in enterocytes and Caco2 cells, make it unlikely that the down-regulating effect of IL-6 and IFNγ on SI are due to a general effect of these cytokines on the state of differentiation of Caco2 cells.

Expression of SI in Normal and Inflamed Crohn’s Intestine in Vivo—Several different approaches were used to examine the possibility that SI gene expression is down-regulated in intestine during inflammation. First, we harvested RNA from normal jejunum, normal ileum, inflamed, and adjacent unin-
Regulation of Sucrase-Isomaltase by Inflammatory Cytokines

Acute phase reactant SAA (Fig. 5, detection assay with an antisense cRNA probe for the positive specific as shown by subjecting the same RNA to RNase protection assay with a radiolabeled SI antisense cRNA. There were no silver grains in the silver grains within enterocytes lining the villi of normal ileum with antisense SI cRNA. There were no silver grains in the villous enterocytes with sense SI probe. Refractile bodies (arrowheads) could be seen over the villous epithelium and lamina propria with antisense cRNA or sense RNA, or when sections were hybridized without any probe; thus, these are nonspecific by definition. There was a patchy decrease in silver grains in uninflamed Crohn's ileum but a marked and diffuse decrease in silver grains in the villous enterocytes of acutely inflamed Crohn's ileum. The difference in density of silver grains between normal ileum and Crohn's ileum was also confirmed by quantitation of the number of silver grains per high power field. For this analysis silver grains per high power field were counted on the base, lower third, middle third, and upper third of 8 different villi from the normal ileum, on 7 different villi from inflamed ileum, and on 8 different villi from uninflamed adjacent ileum from 2 normal and 2 Crohn's donors. The results showed counts of 168 ± 33.6 for normal, 32.0 ± 11.0 for inflamed ileum specimen, and 137.0 ± 37.09 for uninflamed ileum. The difference between normal and inflamed ileum was statistically significant (p < 0.001 by ANOVA and by Tukey's test of multiple comparisons). The difference can also be seen on multiple villi when the sections are shown at lower magnification (Fig. 7b). The decrease in enterocyte-specific SI mRNA in inflamed ileum was specific in that there was an increase in enterocyte-specific α1-AT mRNA in the same specimens (data not shown) and in 3 other individuals with Crohn's disease in

![Fig. 5. RNase protection assays for SI (left panel) and SAA (right panel) mRNA levels in normal jejunum, normal ileum, acutely inflamed Crohn's ileum, and adjacent uninflamed Crohn's ileum.](image)

The migration of the probe is indicated by the left margin in each panel. RNA from Caco2 cells and IL-6-activated Caco2 cells is shown for comparison in the right panel. For each lane, 10 μg of RNA was loaded. The same preparations of RNA were used for both panels. The migration of the labeled probe is shown at the left margin in each case. The migration of undigested probe is indicated by open arrowheads at the right margin. The specific protected RNA fragment is indicated by a solid arrowhead at the right margin of each panel.

![Fig. 4. Effect of inflammatory cytokines on biosynthesis of lactase in Caco2 cells.](image)
our previous studies (13). Taken together, the results of ribonuclease protection assays, immunostaining, and in situ hybridization analyses provide internally consistent evidence for selective and specific decrease in SI gene expression in acutely inflamed Crohn’s ileum. The results of immunostaining and in situ hybridization analysis show that there is a decrease in cell surface SI expression and SI mRNA per enterocyte in the inflamed Crohn’s ileum.

DISCUSSION

The results of this study show that inflammatory cytokines mediate changes in expression of the SI gene in intestinal epithelial cells. IL-6 and IFNγ mediate concentration-dependent decreases, and TNFα mediates a concentration-dependent increase in the biosynthesis of SI in the human intestinal epithelial cell line Caco2. On the other hand, IL-1β has no effect on synthesis of SI in Caco2 cells. The effects of each cytokine on SI synthesis are specific and selective in that they occur in the context of marked changes in expression of other target genes, particularly acute phase genes. This includes cytokine-specific and target gene-specific changes. IL-6 mediates a decrease in SI synthesis at the same time as it mediates an increase in complement proteins factor B, C3, and α1-AT. IL-1β has no effect on synthesis of SI while it mediates an increase in factor B and C3 synthesis. IL-1β also has no effect on synthesis of SI when it is used together with IL-6, even though it has an additive effect on factor B and a synergistic effect on SAA in the same experiment. IFNγ mediates a decrease in SI synthesis at the same time as it mediates an increase in factor B and C4 synthesis. In contrast, TNFα mediates an increase in synthesis of SI and C3, but has little effect on the other genes. The decrease in SI biosynthesis in Caco2 cells activated by IL-6 or by IFNγ could not be attributed to a general effect on cell number or metabolic activity per cell or to heterogeneity within the cell line because sequential immunoprecipitation of the same labeled cell lysates showed an increase in factor B, C3, and α1-AT in the case of IL-6 and an increase in C4 in the case of IFNγ. Similarly the increase in biosynthesis of SI in Caco2 cells treated with TNFα could not be attributed to a general effect or heterogeneity because sequential immunoprecipitation showed no change in synthesis of α1-antichymotrypsin and α1-AT. The changes in SI synthesis are also unlikely to reflect a general effect on the state of differentiation of Caco2 cells because on sequential immunoprecipitation there were no changes in synthesis of two other enterocyte integral membrane proteins that are markers of terminal differentiation in Caco2 cells, lactase and alkaline phosphatase.

In order to examine the possibility that the effects of inflammatory cytokines on SI gene expression in enterocytes are relevant to physiologic and pathophysiologic situations in vivo, we used RNase protection assays and immunofluorescent and in situ hybridization analyses to determine SI gene expression in inflamed ileum from patients with Crohn’s disease. Previous studies have shown that there is increased complement proteins in intestinal loop fluid from patients with Crohn’s disease (29) and increased α1-AT gene expression in enterocytes from acutely inflamed Crohn’s ileum (13). The results of our studies showed that there is a marked decrease in expression of the SI gene in villous enterocytes of acutely inflamed Crohn’s ileum. The decrease could not be attributed to generalized damage to the enterocytes in that there was evidence for substantial increases in SAA and α1-AT gene expression in the same epithelial cells using analysis of cell-specific expression by immunostaining and in situ hybridization. Taken together, these results indicate that there is a specific and selective decrease in SI gene expression in villous enterocytes in one type of intestinal inflammatory condition and that the specificity and selectivity of the effect recapitulates the changes in gene expression elicited in a human intestinal epithelial cell line in tissue culture in response to IL-6 or IFNγ. The data do not prove that IL-6 and/or IFNγ are responsible for the decrease in SI gene expression in vivo. Moreover, the data do not address whether locally- or systemically-derived cytokines are responsible. However, the fact that there is a gradient in the changes in gene expression when comparing the acutely inflamed to the adjacent uninflamed Crohn’s ileum is more consistent with an effect of locally-derived cytokine, or cytokines. Expression of all the cytokines studied here, IL-6, IL-1β, TNFα, and IFNγ, has been detected in the bowel in inflammatory bowel disease (30). Finally, the data do not necessarily imply that TNFα is absent from, or not acting on, villous enterocytes in acutely inflamed Crohn’s ileum. The net effect on SI gene expression here could depend on many factors, including the relative endogenous expression/activity of receptors and soluble receptors/receptor antagonists.

SI is an integral membrane glycoprotein of enterocytes,
which digests dietary disaccharides. Its expression is confined to the small intestine in the adult. Its expression is altered by changes in dietary nutrients and by diabetes mellitus. It is also expressed aberrantly in colonic polyps, adenomas and adenocarcinomas, probably as a result of dedifferentiation to a fetal colonocyte program in these conditions. Several cis-acting DNA binding domains in the upstream flanking region of the SI gene and trans-acting DNA binding proteins that regulate its intestine-specific transcription have been identified in recent studies (26, 31, 32). However, cis-acting elements and transcription factors, which could mediate down-regulation of SI gene expression in response to IL-6 and IFNγ or up-regulation of SI gene expression in response to TNFα, have not been described. There is, however, no a priori reason that these cytokines directly act on SI gene expression. Cross and Quaroni (24) have shown that epidermal growth factor mediates a marked decrease in SI gene expression in Caco2 cells, raising the possibility that the effects of cytokines could be mediated indirectly through epidermal growth factor, transforming growth factor α, or, for that matter, through other related growth factors.

The results of this study do not provide any information about the clinical relevance of depression in SI gene expression in inflammatory bowel disease. It is known that lactose malabsorption is as common in inflammatory bowel disease as it is in the general population (33), but there is relatively little information in the literature about sucrose malabsorption. Nevertheless, the results do raise the possibility that cytokines can mediate an effect that inhibits SI gene expression in Crohn’s disease, in infectious enteritis, or in perturbations of the intestinal flora that result in the local release of cytokines such as IL-6 and IFNγ.

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