Intectin, a Novel Small Intestine-specific Glycosylphosphatidylinositol-anchored Protein, Accelerates Apoptosis of Intestinal Epithelial Cells*

Hidefumi Kitazawa,a,b,c Tamao Nishihara,a,b,d Tapahiro Nambu,* Hitoshi Nishizawa,a,f Masanori Ikawa,* Atsunori Fukuhara,* Toshio Kitamura,* Morihito Matsuda,a,d,h and Iichiro Shimomuraa,f,l,j

From the *Department of Medicine and Pathophysiology, Graduate School of Frontier Bioscience, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, the †Department of Internal Medicine and Molecular Medicine, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, the ‡Department of Hematopoietic Factors, Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo 108-8639, the §Preclinical Research for Embryonic Science and Technology, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, and the ††21st Century Center of Excellence Program, the Japan Society for the Promotion of Science, Tokyo 102-8471, Japan

Intestinal epithelial cells undergo rapid turnover and exfoliation especially at the villus tips. This process is modulated by various nutrients especially fat. Apoptosis is one of the important regulatory mechanisms of this turnover. Therefore, identification of the factors that control epithelial cell apoptosis should help us understand the mechanism of intestinal mucosal turnover. Here, we report the identification of a novel small intestine-specific member of the Ly-6 family, intectin, by signal sequence trap method. Intectin mRNA expression was exclusively identified in the intestine and localized at the villus tips of intestinal mucosa, which is known to undergo apoptosis. Intectin mRNA expression was modulated by nutrition. Intestinal epithelial cells expressing intectin were more sensitive to palmitate-induced apoptosis, compared with control intestinal epithelial cells, and such effect was accompanied by increased activity of caspase-3. Intectin expression also reduced cell-cell adhesion of intestinal epithelial cells.

* This work was supported by The Suzuken Memorial Foundation, The Nakajima Foundation, the Kane Foundation for Life and Socio-Medical Science, The Tokyo Biochemical Research Foundation, the Takeda Medical Research Foundation, Uehara Memorial Foundation, the Takeda Science Foundation, the Novartis Foundation (Japan) for the Promotion of Science, The Cell Science Research Foundation, The Takeda Medical Research Foundation, Uehara Memorial Foundation, The Naito Foundation, the Kanae Foundation for Life and Social Science, Graduate School of Frontier Bioscience, Graduate School of Medicine, Osaka University, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan. Tel.: 81-6-6879-3739; E-mail: ichi@imed2.med.osaka-u.ac.jp.

Intestinal epithelial cells originate from stem cells at the base of the crypt and migrate along the crypt-villus axis toward the intestinal lumen. When intestinal epithelial cells reach the luminal surface at the villus tips, they finally exfoliate into the lumen, and their cell cycle is terminated with a life span of only 3–5 days (1, 2). This constant and rapid turnover of intestinal mucosa is essential for maximal nutrient absorption, adaptation to changes in diet, and repair of mucosal injury (3).

Apoptosis plays an important role in maintaining the physiological integrity of many tissues. In the intestine, apoptosis is a key regulator for the turnover of intestinal mucosa, and apoptotic intestinal epithelial cells have been detected at the villus tips of the small intestine and the colonic luminal surface (4–6). However, the one or more underlying mechanisms of this process have not been elucidated. In this regard, it is important to identify the factors that control intestinal epithelial cell apoptosis to understand the mechanism of intestinal mucosal turnover.

The present study was designed to identify a small intestine-derived protein or secretory protein modulated nutritionally that is involved in the control of intestinal epithelial cell apoptosis. Using the efficient signal sequence trap (SST) method (7), we identified a novel small intestine-specific GPI-anchored protein, intectin, which showed distinct localization at the villus tips of intestinal mucosa and accelerated fatty acid-induced apoptosis of intestinal epithelial cells.

**EXPERIMENTAL PROCEDURES**

**Cloning of Intectin cDNA—Poly(A)+ RNAs were extracted from the small intestinal epithelium of C57BL/6J mice under three feeding conditions; ad libitum, 24-h fasting, and 24-h fasting followed by 24-h feeding, and from the small intestinal epithelium of ad libitum-fed db/db mice. Equal amount of poly(A)+ RNA from each group was pooled and used as the template to synthesize complementary DNA (cDNA). To selectively clone the genes with signal sequence at the N-terminal end of cDNAs, the SST-REX system (signal sequence trap

---

1 The abbreviations used are: SST-REX, signal sequence trap by retrovirus-mediated expression screening system; BSA, bovine serum albumin; DMEM, Dulbecco’s modified Eagle’s medium; FBS, fetal bovine serum; GPI, glycosylphosphatidylinositol; PI, propidium iodide; PIG-A, phosphatidylinositolglycan-class A; PI-PLC, phosphatidylinositol-specific phospholipase C; PNH, paroxysmal nocturnal hemoglobinuria; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline; DIG, digoxigenin; CHO, Chinese hamster ovary cells; RT, reverse transcription; ISH, in situ hybridization.
Intectin, a Novel Intestine-specific GPI-anchored Protein

by gene-transfer-mediated expression screen was introduced as described previously by our laboratory (7). Briefly, cDNA was synthesized from the poly(A)+ RNA by random hexamers, using the SuperScript System (Invitrogen), and then was inserted into BstX1 sites of the pMX-SST vector, using BstX1 adapters (Invitrogen). The ligated DNA was amplified in DH10B cells (Electromax, Invitrogen) to construct an SST-REX library, and a library DNA was prepared using Qiagen plasmid kits (Qiagen). High titer retroviruses representing the SST-REX library were produced using the packaging cell line PlatE and infected to Ba/F3 cells. After 1-day infection period, selection of factor-independent Ba/F3 cells commenced in the absence of interleukin-3, using 96-well multititter plates. The integrated cDNAs were isolated from the interleukin-3-independent Ba/F3 cells by genomic PCR and sequenced (Chimtek) (8).

Animals and Experimental Protocol—C57BL/6J and obese diabetic db/db mice were obtained from Clea Japan (Tokyo) and kept under a temperature for 10 min, diluted in DMEM with 10% FBS, and then plated in 96-well plate (0.5 ml/well) and then placed on BSA pre-coated 24-well plate (0.5 ml). The cells were incubated with ice-cold PBS and fixed with ethanol (70% cold ethanol). After treatment with 1 μg/ml DNase-free RNase A in PBS containing 10 μg/ml PI, the cells were analyzed using a FACSort. The cell cycle distribution was quantified by using FlowJo (Tree Star Inc., Ashland, OR) software.

In Situ Hybridization—According to the instructions supplied by the manufacturer (digoxigenin (DIG) RNA labeling kit (SP6/T7), Roche Diagnostics), DIG-labeled RNA probes were synthesized using intectin mRNA as a template. Following overnight incubation, experiments were designed to determine the expression of intectin mRNA in the small intestine. The absence of black and purple signals. Some specimens were counterstained with hematoxylin.

Viability Assay—The cells (5 × 10^5)/well) were treated with cell lysis buffer (RIPA buffer), and centrifuged (10 min, 15,000 × g, 4 °C) to remove debris. After determination of protein concentration using the BCA protein quantification formula provided by the manufacturer.

Cell Aggregation Assay—Cell aggregation assay was performed as described previously (10). Parental Chinese hamster ovary (CHO) cells and interleukin-3-transfected CHO cells (CHO-intectin) were washed with PBS and incubated with 0.125% trypsin and 0.5% EDTA at 37 °C for 3 min. Dispersed cells were suspended in Hanks’ balanced salt solution (1 × 10^6 cells/ml) and then placed on BSA pre-coated 24-well plate (0.5 ml). The cells were incubated under continuous shaking at 70 rpm for the indicated periods of time. The reaction was stopped with the addi-
tion of an equal volume of 4% paraformaldehyde. Samples were evaluated by counting single cells and aggregates.

Statistical Analysis—Data are expressed as means \pm S.E. Statistical analyses were performed with unpaired \textit{t}-tests. A \textit{p} value less than 0.05 denoted the presence of a statistically significant difference.

RESULTS

Identification of Intectin as a Small Intestine-derived Nutritionally Modulated Membrane Protein—We previously developed an efficient SST method using retrovirus-mediated gene transfer (7). To identify small intestine-derived nutritionally modulated membrane protein or secretory protein, we conducted this SST method using the pooled poly(A)\(^+\) RNA from the small intestinal epithelium of mice at \textit{ad libitum}, fasted and refed conditions, and obese diabetic \textit{db/db} mice. We screened and sequenced 1224 clones. Four clones were selected as unknown proteins with signal sequence or transmembrane region. The mRNA of one clone was exclusively expressed in the small intestine among various tissues of mice as revealed by Northern blotting analysis and quantitative RT-PCR (Fig. 1, \textit{A} and \textit{B}). This clone and its full-length cDNA were selected and named intectin. The nucleotide sequence and deduced 111-amino acid sequence of intectin are shown in Fig 1\textit{C}. A hydrophobicity plot revealed a 20-residue N-terminal and a 13-residue C-terminal signal peptide (Fig. 1, \textit{C} and \textit{D}) (11). A potential GPI-anchoring site was identified at Asn-88 based on the published GPI consensus sequences (Fig. 1\textit{C}). A Basic Local Alignment Search Tool (BLAST) search revealed that intectin sequence is identical to NM_025929 (GenBank\textsuperscript{T\textregistered}), whose function is totally unknown, and that intectin has a Ly-6 domain (Fig. 2). All cysteine residues highly conserved among Ly-6 family are present in intectin. The amino acid similarity of intectin to other Ly-6 family is not high, with homology of only 30–40% (Fig. 2).

We established a stable cell line that expressed intectin by transfecting the intectin-FLAG gene into a normal intestinal epithelial cell line, IEC-6. Expression of intectin protein in intectin-transfected IEC-6 (IEC-intectin) and mock-transfected IEC-6 (IEC-mock) are shown in Fig. 3\textit{A}. IEC-intectin cells expressed intectin protein in the cell membrane fraction, and intectin protein was not detected in the cytosolic fraction (Fig. 3\textit{A}). These results suggest that intectin can be considered a membrane-associated protein. As described above, intectin protein has a predicted GPI-anchoring site in Asn-88. To clarify whether intectin is a GPI-anchored protein, we treated the cells with phosphatidylinositol-specific phospholipase C (PI-PLC) exhibiting activity to excise GPI-anchored region. Without PI-PLC treatment, IEC-intectin cells exhibited higher FITC signal on cell membrane, compared with IEC-mock cells (Fig. 3\textit{B}). PI-PLC treatment of IEC-intectin cells decreased the signal to the level of IEC-mock cells (Fig. 3\textit{B}). The same results were observed in FLAG-tagged intectin-transfected CHO cells as well as FLAG-tagged CD59, a known GPI-anchored protein, transfected CHO cells (data not shown). These results indicate that intectin is a GPI-anchored protein.

Exclusive Expression of Intectin in the Small Intestine—Analysis of various tissues of 6-week-old C57BL/6J mice by Northern blotting and quantitative RT-PCR showed that intectin mRNA was expressed exclusively in the small intestine (Fig. 1, \textit{A} and \textit{B}). Unlike other known members of the Ly-6 family, which are highly expressed in peripheral blood leuko-
cytes and in lymphoid organs, intectin mRNA expression was not detected in the bone marrow, WR19L (mouse T cell line), WEHI3 (mouse macrophage-like cell line), and BaF3 (mouse pro-B cell line) cells by quantitative RT-PCR (data not shown). In situ hybridization was performed to determine the distribution of intectin mRNA in the intestine. Remarkably, a strong signal was detected only at the villus tips of the duodenum, jejunum, and ileum (Fig. 4, B, E, H, and N), and only a weak signal was detected in the colon (Fig. 4K), and a trace signal was found in the stomach and esophagus (data not shown). No signal was detected in all other tissues examined, including the brain, spleen, heart, lungs, eyes, lymph nodes, thyroid, skeletal muscles, thymus, liver, WAT, BAT, pituitary, adrenals, urinary bladder, gallbladder, and testes, by in situ hybridization (data not shown). Thus, intectin mRNA was exclusively expressed in the villus tips of the epithelial cells of the mouse small intestine. In this regard, Groos et al. (6) reported the exclusive presence of apoptotic cells at the villus tips in human and rat

Fig. 2. Amino acid alignments between intectin and other mouse Ly-6 proteins. The amino acid sequence of intectin is aligned with other known mouse Ly-6 proteins. The highly conserved 10 cysteines in mouse Ly-6 proteins are indicated by **bold boxed letters**.

Intectin | MRT-HELWWLPLII--GSAAQLKLE [C] HE C SG---IED C YKPCT C SSQSLY C 45
my-6A | MDSYTBSCSLLLWLLAVCAERQGLKLE [C] YQ C YQQFETS C PS-I7 C RPQGV C 53
my-6C | MDSYTBSCSLLLWLLAVCAERQGLKLE [C] YQ C YQQFETS C PA-V7 C RASQGF C 53
ThB | MKTA------LLVLVLVATAVSALR K HV C THSAN--- C KNQPV C PSNFFY C 45
TSA-1 | MSATSNMVRLVLLAALGLMEQVHSL M [C] FS C TDQKNNIN C LWPSV C QEDH Y C 54

Intectin | LNNYTFP-QQQTVTKK---- C NVT C P-DINHTA------NSKSS CC NTDL C NSA 87
my-6A | VDQEAAYVDQSQTKV-NNL C LPI C EPNNESMLGTXVMTC CC QEDL C NVA 107
my-6C | IAONELDAS RKKLTKQ---- C LDF C FAPGF----FKNFIRETS CC SEDL C NAA 104
ThB | KTVSVFVPLNGNLYRKE---- C ANS C TDYSQGCHVSQGSEV-QH CC QTDL C NER 93
TSA-1 | ITLSAAGFQNVGLYTINHG C SPI C PS---ENVLNLGVASVNSY CC QSSF C NFA 107

Intectin | RNLHVS-WG------LL-ALG---LVYILS Q 111
my-6A | VEAGGSTMAGVLLLFSLSYLQTL T 134
my-6C | VPTAGSTMGAVLLLFSLSYLQTL 131
ThB | LVSAGRGA---LLS----SVILGLATSLSLLTVMACL 127
TSA-1 | AAGLGLASIP----LL-GLG-LLGLALLQLP 136

**Fig. 3. Expression of FLAG-tagged intectin on IEC-6 cell surface and sensitivity to PI-PLC.** A, location of intectin protein. The membrane fraction and cytosolic fraction of IEC-mock cells and IEC-intectin cells were subjected to Western blotting using anti-FLAG antibody. FLAG-tagged intectin protein is indicated by the solid arrowhead. Nonspecific bands are indicated by the open arrowheads. B, IEC-mock cells and IEC-intectin cells were treated with (dotted line) or without (solid line) PI-PLC for 1 h at 37 °C. Then, the cells were stained with FITC-conjugated anti-FLAG antibody and analyzed by flow cytometry.
Intectin, a Novel Intestine-specific GPI-anchored Protein

Intectin Expression Is Modulated by Nutritional Status—We next examined the nutritional regulation of intectin mRNA expression in vivo (Fig. 5A). Intectin mRNA expression in the small intestine was significantly decreased by 24-h fasting and restored to basal level by 1- to 2-h refeeding (Fig. 5A). These results indicate that intectin mRNA expression is modulated by nutritional stimuli. This conclusion was supported by our finding in db/db mice, whose daily food intake is 1.5- to 2-fold higher than that of age-matched C57BL/6J mice (data not shown); intectin mRNA expression in the small intestine of 6-week-old db/db mice was significantly higher than ad libitum-fed C57BL/6J mice (Fig. 5B).

Involvement of Intectin in the Rapid Turnover of Intestinal Mucosa—Based on its specific expression at the villus tips of the intestine and active nutritional regulation, we hypothesized that intectin could be involved in the rapid turnover of intestinal mucosa. To test our hypothesis, we first compared the viability of IEC-intectin cells and IEC-mock cells. Cell viability assay was performed after 12-h treatment with palmitate and then restored to basal level by 1- to 2-h refeeding (Fig. 5A). These results indicate that intectin mRNA expression is modulated by nutritional stimuli. This conclusion was supported by our finding in db/db mice, whose daily food intake is 1.5- to 2-fold higher than that of age-matched C57BL/6J mice (data not shown); intectin mRNA expression in the small intestine of 6-week-old db/db mice was significantly higher than ad libitum-fed C57BL/6J mice (Fig. 5B).

Palmitate significantly decreased the G1 population, whereas it increased the sub-G1 population and the cell death fraction, including apoptotic cells. These palmitate-induced changes in the cell cycle toward cell death were more pronounced in IEC-intectin cells compared with IEC-mock cells. For example, the majority of IEC-mock cells were in the G2 or G1 phase, while the majority of IEC-intectin cells were in the sub-G1 phase when these cells were incubated in the presence of 50 or 100 μM palmitate (Fig. 6B).

To further examine the possible involvement of intectin in the rapid turnover of intestinal mucosa through cell death and especially through apoptosis, we measured caspase-3 activity in palmitate-treated IEC-intectin cells and IEC-mock cells (Fig. 6C). Because activation of caspase-3 is a common downstream effector of diverse apoptotic pathways (13), we measured cleavage of colorimetric substrate, specific to caspase-3. Low dose palmitate significantly increased caspase-3 activity in IEC-intectin cells compared with in IEC-mock cells. Taken together, the above results suggest that intectin expression causes activation of caspase-3, which in turn induces apoptotic death of IEC-6 cells.

Intectin Reduces Cell-to-Cell Adhesion—Previous studies suggested that some members of the Ly-6 family are involved in cell cycle regulation, including apoptosis, we measured caspase-3 activity in palmitate-treated IEC-intectin cells and IEC-mock cells. For example, the majority of IEC-mock cells were in the G2 or G1 phase, while the majority of IEC-intectin cells were in the sub-G1 phase when these cells were incubated in the presence of 50 or 100 μM palmitate (Fig. 6B).

To further examine the possible involvement of intectin in the rapid turnover of intestinal mucosa through cell death and especially through apoptosis, we measured caspase-3 activity in palmitate-treated IEC-intectin cells and IEC-mock cells (Fig. 6C). Because activation of caspase-3 is a common downstream effector of diverse apoptotic pathways (13), we measured cleavage of colorimetric substrate, specific to caspase-3. Low dose palmitate significantly increased caspase-3 activity in IEC-intectin cells compared with in IEC-mock cells. Taken together, the above results suggest that intectin expression causes activation of caspase-3, which in turn induces apoptotic death of IEC-6 cells.

Intectin Reduces Cell-to-Cell Adhesion—Previous studies suggested that some members of the Ly-6 family are involved
Fig. 6. Effects of palmitate on apoptosis of IEC-6 cells. A, effect of palmitate on viability of IEC-6 cells. IEC-intectin and IEC-mock cells were treated with palmitate. After 12-h treatment, the cells were treated with WST-8 and incubated for 1 h at 37 °C. The optical densities were measured at 450 and 650 nm. Values are expressed as mean ± S.E. (n = 3). *, p < 0.05. B, effect of palmitate on cell cycle. The cells were incubated with 0, 25, 50, or 100 μM palmitate for 12 h, and fixed with 70% cold ethanol. Then the samples were treated with 10 μg/ml RNase, stained with 10 μg/ml propidium iodide, and analyzed by flow cytometry. The sub-G1 range is indicated by a horizontal bar above which the percentage of nuclei
Intectin, a Novel Intestine-specific GPI-anchored Protein

42873

in cell-cell adhesion (14, 15). Therefore, we conducted a cell aggregation assay using parental CHO cells (CHO) and CHO cells stably expressing intectin (CHO-intectin). Intectin expression significantly attenuated CHO cell aggregation (Fig. 6D), suggesting that intectin expression weakens the cell-cell adhesion probably to promote the exfoliation of intestinal epithelial cells.

DISCUSSION

The intestinal mucosa is continuously exposed to various toxic factors, including enteropathogenic microorganisms and food antigens. It also has to face potent digestive enzymes present in the lumen secreted from the liver and pancreas. In such harmful environments, the intestinal mucosa exhibits a rapid turnover, which serves to maintain the essential functions of the intestine and the integrity of the intestinal wall. The regulation of this rapid turnover of intestinal mucosa is complex and controlled by several factors. Apoptosis is one important factor that determines this process (16, 17). Therefore, identification of the regulatory factors of intestinal mucosal apoptosis should help us understand the mechanism of intestinal mucosal turnover.

In the present study, we identified intectin using an efficient SST technique. Although the precise physiological significance of intectin has yet to be determined, several important features were defined in the present study. 1) Intectin is a new member of GPI-anchored Ly-6 family; 2) intectin mRNA is exclusively expressed in the villus tips of the small intestine, which are known to undergo apoptosis; 3) intectin mRNA expression is influenced by nutritional changes; 4) intectin expression increases the sensitivity of intestinal epithelial cells to palmitate-induced apoptosis; and 5) intectin expression significantly attenuates cell aggregation. These findings suggest that intectin might modulate nutrition-dependent apoptosis, weaken cell-cell attachment, and promote the dying cells to exfoliate into the lumen at the final stage of intestinal mucosal turnover.

The intectin gene is located on murine chromosome 15, similar to murine Ly-6 genes (18). The intectin protein is composed of 111 amino acid residues and has a Ly-6 domain, which is defined by a distinct disulfide-binding pattern between 10 cysteine residues. Furthermore, intectin protein has N-terminal and C-terminal signal sequences, and the result of PI-PLC treatment of intectin-expressing cells indicated that intectin was a GPI-anchored protein, a hallmark of Ly-6 family. These findings indicate that intectin is a novel member of the Ly-6 family. Previous studies suggested the involvement of Ly-6A/E in T-cell activation (19, 20) and development (21). Ly-6A/E and Ly-6C have been shown to regulate cell adhesion (14, 15), but the in vivo function of Ly-6 family are unknown with the exception of CD59 and urorokinase-type plasminogen activator receptor. CD59 functions as a membrane inhibitor of reactive lysis, and failure to express CD59 is related to the pathogenesis of paroxysmal nocturnal hemoglobinuria (PNH). In PNH, acquired somatic defect of the PIG-A gene results in a defect in GPI-anchored proteins expression, including CD59, on the cell surface, making blood cells more susceptible to host complement-mediated lysis (22, 23). These patients are more susceptible to leukemia (24) due to resistance to apoptosis caused by PIG-A gene mutations, suggesting that some of GPI-anchored proteins are important in modulating apoptosis (25). Considered together with the results of the present study, it is conceivable that intectin, a member of the GPI-anchored Ly-6 family proteins, is involved in the regulation of intestinal epithelial cell apoptosis.

Induction of apoptosis by palmitate has been reported in various cells, including pancreatic β-cells (26), cardiomyocytes (27), and hematopoietic cells (28). In the present study, we demonstrated that palmitate induced apoptosis of intestinal epithelial cells. Furthermore, recent studies reported nutritional modulation of intestinal mucosal apoptosis (29–31).

Raab et al. (29) showed that high energy diet and purines in the diet induced intestinal mucosal apoptosis. In addition, Sukhotnik et al. (30) reported that exposure to low fat diet decreased intestinal epithelial cell apoptosis in a rat model of short bowel syndrome. These reports implicate nutrient-derived fat should induce apoptosis of intestinal epithelial cells. Groos et al. (31) also showed that apoptosis of intestinal epithelial cells at the villus tips was markedly reduced in subjects receiving total parenteral nutrition over 2 weeks compared with enterally nourished subjects, suggesting that food components seem to influence apoptosis of intestinal epithelial cells. The precise molecular mechanism that regulates apoptosis of intestinal mucosa in response to nutritional conditions has not yet been established. Since intectin mRNA expression was restored as fast as 1 h after refeeding following 24-h fasting, intectin expression is more likely regulated by food-related physical stimuli rather than by endocrine factors such as insulin. Palmitate is a major fatty acid in fat diet and is probably involved in fat diet-induced apoptosis of intestinal mucosa. Our finding that intectin accelerated palmitate-induced apoptosis of intestinal epithelial cells suggests that diet-induced expression of intectin might mediate this diet-induced apoptosis of intestinal mucosa.

Apoptotic cells are observed at the villus tips of intestinal mucosa. In certain inflammatory conditions, such as celiac disease, nematode infections, and graft-versus-host disease, the numbers of apoptotic nuclei were increased in villus epithelial cells (32–35). In addition, the involvement of dysregulation of the apoptotic process in intestinal epithelial cells in inflammatory bowel diseases and colon cancer has also been suggested (36, 37). These studies indicate that apoptosis plays some role in pathological conditions as well as in physiological turnover of villus epithelial cells. In this regard, whether intectin is involved in the pathology of inflammatory bowel diseases or colon cancer remains to be elucidated.

In conclusion, we described in this study the identification of intectin, a novel intestine-specific GPI-anchored protein. This protein enhanced palmitate-induced apoptosis of intestinal epithelial cells.

Acknowledgments—We thank the members of Shimomura’s laboratory for the helpful discussions. We thank Drs. Yusuke Maeda and Taro Kinoshita (Osaka University) for kindly providing the pME-puro-FLAG-CD59 vector and for the helpful suggestions. We also thank Drs. Rikinari Hanayama and Shigekazu Nagata (Osaka University) for kindly providing the expression plasmid pEF-BOS and for the invaluable suggestions.

REFERENCES

1. Potten, C. S. (1992) Cancer Metastasis Rev. 11, 179–195
2. Potten, C. S., and Allen, T. D. (1977) J. Ultrastruct. Res. 60, 272–277
3. Willingham, R. C. (1976) N. Engl. J. Med. 298, 1444–1450
4. Jones, B. A., and Gore, G. J. (1997) Am. J. Physiol. 273, G1174–G1188
5. Potten, C. S. (1997) Am. J. Physiol. 273, G253–G257

containing the sub-G, amount of DNA is indicated. C, IEC-intectin and IEC-mock cells were incubated with 0, 25, 50, 100, or 200 μM palmitate for 12 h. Caspase-3 activity was measured as described under “Experimental Procedures.” Values are expressed as mean ± S.E. (n = 4), *, p < 0.05, compared with IEC-mock cells. D, cell aggregation assay. Parental CHO cells and CHO-intectin cells were subjected to aggregation assay as described under “Experimental Procedures.” Cells remaining as single cells were counted at indicated periods of time. The number of cells present in aggregates was also counted. Values are expressed as mean ± S.E. (n = 4). *, p < 0.05, compared with parental CHO cells.
Intectin, a Novel Intestine-specific GPI-anchored Protein

6. Groos, S., Reale, E., and Luciano, L. (2003) Anat. Rec. 272, 503–513
7. Kojima, T., and Kitamura, T. (1999) Nat. Biotechnol. 17, 487–490
8. Nishizawa, H., Shimomura, I., Kishida, K., Maeda, N., Kuriyama, H., Nagareta, H., Matsuda, M., Kondo, H., Furuyma, N., Kihara, S., Nakamura, T., Tsuchino, Y., Funahashi, T., and Matsuzawa, Y. (2002) Diabetes 51, 2734–2741
9. Uchida, E., Mizuguchi, H., Ishii-Watabe, A., and Hayakawa, T. (2002) Biol. Pharmaceut. Bull. 25, 891–897
10. Whittard, J. D., and Akiyama, S. K. (2001) Exp. Cell Res. 263, 65–76
11. Kyte, J., and Doolittle, R. F. (1982) J. Mol. Biol. 157, 105–132
12. Johnson, R., Lancki, D. W., and Fitch, F. W. (1993) J. Immunol. 151, 2986–2999
13. Green, D. R. (2000) Cell 102, 1–4
14. Bamezai, A., and Rock, K. L. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 4294–4298
15. Hanninen, A., Jaakkola, I., Salmi, M., Simell, O., and Jalkanen, S. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 6898–6903
16. Potten, C. S., Wilson, J. W., and Booth, C. (1997) Stem Cells 15, 82–93
17. Hall, P. A., Coates, P. J., Ansari, B., and Hopwood, D. (1994) J. Cell Sci. 107, 3569–3577
18. LeClair, K. P., Rabin, M., Neshitt, M. N., Pravtcheva, D., Ruddle, F. H., Palfree, R. G., and Boothwell, A. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 1638–1642
19. Flood, P. M., Dougiberty, J. P., and Ron, Y. (1990) J. Exp. Med. 172, 115–120
20. Lee, S. K., Su, B., Maher, S. E., and Boothwell, A. L. (1994) EMBO J. 13, 2167–2176
21. Bamezai, A., Pfaller, D., Bereczkovszkai, A., McGrew, J., Higgins, K., Lacy, E., and Rock, K. L. (1995) J. Immunol. 154, 4233–4239
22. Kinoshita, T., Ohishi, K., and Takeda, J. (1997) J. Biochem. (Tokyo) 122, 251–257
23. Rosse, W. F., and Ware, R. E. (1995) Blood 86, 3277–3286
24. Horikawa, K., Nakamura, H., Kawaguchi, T., Iwamoto, N., Nakamura, S., Kagemoto, T., and Takatsuki, K. (1997) Blood 90, 2716–2722
25. Brodsky, R. A., Vara, M. S., Barber, J. P., Medof, M. E., and Jones, R. J. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 8756–8760
26. Shimabukuro, M., Zhou, Y. T., Levi, M., and Unger, R. H. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 2498–2502
27. Sparagna, G. C., Hicken-Bick, D. L., Buja, L. M., and McMillin, J. B. (2001) Antioxid. Redox. Signal. 3, 71–79
28. Paumen, M. B., Ishida, Y., Muramatsu, M., Yamamoto, M., and Honjo, T. (1997) J. Biol. Chem. 272, 3324–3329
29. Raab, S., Leiser, R., Kemmer, H., and Claus, R. (1998) Metabolism 47, 1105–1111
30. Sukhotnik, I., Shiloni, E., Krausz, M. M., Yakirevich, E., Sabo, E., Mogilner, J., Coran, A. G., and Harmon, C. M. (2003) J. Pediatr. Surg. 38, 1182–1187
31. Groos, S., Reale, E., Hunefeld, G., and Luciano, L. (2003) J. Surg. Res. 109, 74–85
32. Moss, S. F., Attia, L., Scholes, J. V., Walters, J. R., and Holt, P. R. (1996) Gut 39, 811–817
33. Hyoh, Y., Nishida, M., Tegoshi, T., Yamada, M., Uchikawa, R., Matsuda, S., and Ariizono, N. (1999) Parasitology 119, 199–207
34. Hysh, Y., Ishizaka, I., Hori, T., Fujisawa, A., Tegoshi, T., Yamada, M., and Ariizono, N. (2002) Gut 50, 71–77
35. Stuber, E., Buschenfeld, A., von Freier, A., Arendt, T., and Folsch, U. R. (1999) Gut 45, 229–235
36. Ruzin, M. F., Seidman, E. G., and Lentze, M. J. (2002) J. Pediatr. Gastroenterol. Nutr. 34, 254–260
37. Shanmugathan, M., and Jothy, S. (2000) Pathol. Int. 50, 273–279