Background: Performance of mucosal epithelial barrier is modified by numerous agents that exert effects on mucin-Mucin Binding Protein (MBP) complex. The aim of the studies described was to determine the nature of the damage or modification of oral mucous barrier by the short-term exposure to ethanol. Methods: Culture of rat buccal mucosa in the presence of ethanol and $[^3H]$-labeled proline and palmitate revealed substantial decrease in MBP synthesis and the release of MBP to the medium. The radioscanning of the samples prepared from the culture medium and the apical epithelial membranes subjected to SDS-PAGE and western blotting disclosed that the released, water soluble 97kDa MBP glycopeptide was labeled with proline and palmitate. When the experiments were conducted in the presence of 5mM EDTA, the GPI-PLD inhibitor, the majority of radiolabeled MBP remained in the membrane-bound form and was extractable with Triton X-114. The results on the purified GPI-linked MBP degradation by serum enzyme, by the saliva containing serum transudate, and the suppression of the process by inclusion of GPI-PLD-specific inhibitor support our contention that membrane MBP is released to medium by GPI-PLD-like activity. Results: The release of MBP from apical epithelial surfaces was induced by depletion of mucin and the presence of serum-derived GPI-PLD in the tissue homogenate. Strong likelihood exists that under in situ conditions ethanol-induced transudation of serum to saliva provides the vehicle for the transfer of GPI-PLD activity to salivary contents. Defacement of the oral surfaces from mucous barrier signals prospect of lumenal agent influence on the unprotected epithelial exterior, and allows ingestion of microbes and untoward acting substances into the organism.

Key words: mucosal epithelial barrier; mucin, mucin binding protein, phospholipase D

INTRODUCTION

Consensus is that body surfaces covered with epidermis inherently limit infection, but the mucosal epithelia due to absorptive functions are precluded from being impervious to pathogens. Mucosal surfaces of oral, tracheobronchial, gastrointestinal and urogenital systems are mainly recognized for their potential to shape our
physiology through bacteria-specific transcriptional responses. These responses are considered as the primary etiologic factors in the development of disease and the relation of the pathogen to mucosal inflammatory changes (1-3). Consequently, the immune system at these epithelial surfaces has become the main paradigm for the study of local and systemic immune responses (1,4-6). While on the contrary, the significance of the mucosal mucous barrier that abates indiscriminate contact of mucosal surfaces with lumenal contents remains unresolved. The instances of mucosal barrier penetration by microbes and profound cellular response to infection are not interpreted as barrier malfunction but as the exclusive reliance of the system on stimulated immunoresponses. On that account, the function of the elaborate mucosal mucous barrier is basically recognized for its buffering and lubricating property, and its tenacity to adhere to the apical surfaces is not considered as an important asset, but rather an impediment to oral immunization (2,7,8). The exclusion of mucous barrier as the primary protective device available to the host is further obscured by the lack of common apperception that the mucosal mucous barrier serves the same purpose as epidermis. Just as microscopy and animalcules harvested from teeth helped breaking the mindset on infections in the 17th century (9), a closer look at mucosal protection is crucial to our endeavors in 21st century.

With the fundamental tenet that human and animal organism ought to have a well developed protective ensemble for screening and modulating signals transferred through the mucosal epithelia, we investigated the principles that govern optimal function of the mucous barrier and the factors causing its breakdown (10-13). Our findings on the chemistry of mucosal mucous barrier of the apical epithelial surfaces demonstrated that the explicit boundary is formed when specific oligosaccharides of mucin are binding with the integral membrane protein of apical epithelial surfaces (12-14). The assembled complex (barrier) represents the distinct entity that allows the specific and appropriate signals and nutrients to penetrate, but stops the contact and ingress of microbes and indiscriminate access of untoward acting substances. The support for this theory has accumulated from investigations of the complex in the context of evaluation of drugs with the potential to heal the injured epithelium (gastric ulcers) (15-17), and those that cause gastric pathology (18-20). Studies with sulglycotide, consisting of sulfated carbohydrate chains, an active gastroprotective agent used in gastric and duodenal ulcer treatment, provided direct evidence as to the role of carbohydrate-specific sites in shielding epithelial surface from microbial contact (19-21), from binding lipopolysaccharides to the MBP, and restoring control of events associated with cellular proliferation and efficacy of signaling of the mucosal growth factors (16,17,22).

In contrast, destruction of microbe-impervious mucosal barrier complex by the release of mucin was accompanied by binding H. pylori LPS to MBP (20) and its consequences manifested in the release of proinflammatory cytokines (23). The role of mucin carbohydrate-specific oligosaccharides in protection of gastric epithelia was
demonstrated in the studies in vivo with rats subjected to chronic alcohol feeding (12). Alcohol-induced changes in mucin synthesis and glycosylation were reflected in measurable defacing of the mucosal barrier from epithelial surfaces, and the reduction of interaction between mucin and MBP. On the average, the carbohydrate-specific interaction between mucin and MBP decreased to 30% of the controls and in several animals it was reduced to zero. These studies, performed on individual animals and the samples of mucin collected in sequence over 9 weeks, provided first evidence that the specific retention of mucin and the protection of mucosal surfaces is controlled by the posttranslational modification of the molecule. Prerequisite is the generation of carbohydrate determinants that capacitate specific interaction of mucin with membrane anchored MBP, and thus form protective barrier for the epithelium (13). Further studies on the structure of the mucous barrier and oral mucosal defenses revealed that the protective complex was disrupted by other means than mucin carbohydrate modification. It was observed that during short-term tissue culture of buccal mucosa, MBP was released to the medium.

In the studies described here, we provide evidence that oral mucosa mucous barrier is released from the epithelial cells with the aid of phospholipase D-like activity. Just as described for serum-derived GPI-PLD, the release of MBP from apical epithelial surfaces is stimulated by serum and inhibited by GPI-PLD-specific inhibitors. We propose here that GPI-specific PLD, ubiquitously present in serum, may often contribute to the demise of oral mucosal mucin barrier, and by defacing oral epithelial surfaces, allow ingestion of microbes and untoward acting substances into the organism.

MATERIALS AND METHODS

Materials, Chemicals and Reagents

Most of the materials used were from Sigma Chemical Co., St Louis, MO. The following materials were purchased from other sources: [9,10^3]H]palmitic acid, [3H]proline and Econofluor scintillation solution were from New England Nuclear, Boston MA, Glyco-Stain, prestained molecular weight standards, column chromatography media were from Bio-Rad, Richmond, CA, Centricon microconcentrators were from Amicon, Beverly, MA, high performance thin layer chromatography plates were from Merck, Rahway, NJ.

Buccal mucosa cells labeling and isolation of Triton X-100 insoluble apical membranes

Rat buccal mucosa cells were harvested from 1 cm^2 excised tissue, or by gentle scraping the surface of the buccal mucosa area with cell scraper. The material was suspended in Dulbecco’s modified Eagle’s medium (DMEM). Ten minutes before labeling, medium was changed to DMEM minus proline. Cells were then labeled for 3h in this medium (250 µl per dish) with addition of
50 µCi of [3H]proline, or 250 µCi [3H]palmitate in the presence of 0-1.5 M ethanol. The culture medium was removed, filtered through 0.2 µM filter, dialyzed and frozen. The buccal mucosa cells were rinsed several times with complete DMEM to remove free radiolabel. The cells were lysed on ice with 1 ml of 25 mM HEPES, pH 7.5, 0.15 M NaCl, 1% (v/v) Triton X-100 and protease inhibitors cocktail (1 mM phenylmethylsulfonyl fluoride, 100 kallikrein U/ml of aprotinin, 5 µg/ml leupeptin and 5 µg/ml pepstatin) for 20 min (13,24). Lysates and insoluble material were collected and centrifuged for 2 min at 12,000 rpm at 4°C. Where indicated the cell lysis and the following sucrose gradient were performed in the presence of 5mM EDTA. The insoluble membranes were homogenized using eight strokes of a Dounce homogenizer and brought to 40% sucrose using 80% sucrose in the lysis buffer without Triton X-100. A step sucrose gradient (5-30%) in the same buffer was layered over the membranes and centrifuged 19 hours at 39,000 rpm (200,000Xg) at 4°C in Beckman SW41 rotor. Fractions (1 ml each) were collected, diluted to 10% sucrose and the insoluble membranes recovered by centrifugation. The fragments of apical membranes were recovered from the tube containing 10 %sucrose. The material was subjected to Triton X-114 extraction and phase separation (13). In some experiments, the membranes were isolated as described in (14) and lysed in 2 ml of lysis buffer containing 1% precondensed Triton X-114 or 60 mM octyl glucoside instead of Triton X-100. The materials from the culture medium and lysed cells were subjected to SDS–PAGE, western blotting, staining for glycoproteins and proteins and scanning for the detection of radiolabeled proline and palmitate (13).

**Measurement of glycosylphosphatidylinositol-specific phospholipase D (GPI-PLD) activity. Release of MBP from apical membranes of rat buccal mucosa**

Samples of purified MBP (13,14), the Triton X-114 insoluble apical membranes, and the extracts of the membrane with Triton X-114 in 100 µl of 50 mM TRIS-maleate pH 7.0 buffer were incubated at 37°C for 1h with 10 µl of rat saliva or 10 µl rat serum, or 10µl of 100 000xg supernatant derived from the preparation of the rat buccal mucosa membranes. Where indicated, the incubation was performed in the presence of protease inhibitors and 5mM EDTA (25-27). Following incubation, the samples were subjected to extraction with Triton X-114, and the water-soluble and the detergent-soluble phase was subjected to SDS-PAGE, western blotting, and detection of MBP with glycoprotein detecting stain, and radioscanning.

**Binding Assay**

To examine interaction of MBP with mucin, the purified GPI-MBP and the water soluble MBP (0.2 µg) was incubated with salivary mucin (ranging from 1-100-fold excess) in a buffer consisting of 150 mM NaCl, 1 mM CaCl₂, 10 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES, pH 7.0) and 0.1 mM phenylmethylsulfonyl fluoride (PMSF) in a final volume of 20 µl. After incubating for 60 min at 37°C, 50% sucrose solution in an electrophoresis buffer was added to the sample (0.5 volume) and samples loaded on 5% nondenaturing polyacrylamide gel. The proteins were separated by running the gel for 20 h at a constant 15 mA. Proteins were transferred to nitrocellulose and radioactivity scanned using Berthold Linear Analyzer.

**Proteolytic degradation**

Samples of radiolabeled MBP were incubated with 10µl of fresh rat saliva, or buccal mucosa extract, or pronase (13). The GPI-MBP was recovered by the extraction with Triton X-114, and the water-soluble products were subjected to SDS-PAGE and radioscanning. Where indicated, the
water-soluble fragments were extracted with water saturated mixture of chloroform/methanol (1:1, v/v) or butanol, and the extract subjected to thin layer chromatography and scanning. For the controls, the incubation was performed in the presence of protease inhibitors consisting of 1 mM phenylmethylsulfonyl fluoride, 100 kallikrein U/ml aprotinin, 2 µg/ml leupeptin, and 2 µg/ml pepstatin and 5 mM EDTA.

**Nitrous Acid deamination of GPI-MBP and the water-soluble palmitate labeled MBP**

Detergent-depleted samples were dialyzed against water, reduced in volume in a concentrator and extracted twice with 3-4 volumes of chloroform/methanol (2:1, v/v) saturated with water (13). To the samples adjusted to 1.2 ml of 0.1 M sodium acetate (pH 3.5) was added 0.3 ml of 1M sodium nitrite, and the pH of the solution adjusted to 4.0 by the addition of 6 N HCl. (28). After incubation of the mixtures at 50°C for 4 h, the lipid product was removed by extraction with chloroform/methanol mixture described above, and with chloroform. The combined organic extracts were evaporated and subjected to thin layer chromatography. For control, the deamination reaction in the presence of 1 M sodium nitrate was employed.

**Thin-layer chromatography**

Thin layer chromatography was performed on 10x20 and 10x10 cm Silica Gel 60 plates. For the separation of phospholipids, the plates were activated at 80°C for 1 hour. For the glycan chromatography, the plates were used without activation. Development was with solvent mixtures consisting of hexanes/2-propanol (95:5, v/v), hexanes/diethyl ether/acetic acid (60:30:1, v/v/v), and chloroform/pyridine/formic acid (50:30:7, v/v/v). The positions of the radioactive components were identified by radioscanning using Berthold Radioactivity Analyzer. The scanning was routinely performed for 10 min/lane or 3-16 hours to scan the entire plate.

**SDS-PAGE chromatography and radioscanning**

The samples were chromatographed on 5, 7.5 and 10% SDS-PAGE, electroblotted to nitrocellulose paper, scanned for radiolabeled proline and palmitate in Berthold Linear Analyzer and stained for carbohydrate with Glyco-Stain. After transfer, the PAGE gels were developed with Coomassie stain.

**RESULTS**

Oral epithelial surfaces, just as gastric mucosal apical surfaces display strong affinity for mucin. The staining of buccal mucosa with mucin antibodies shows mucin cloaked around single mucosal cells, but in cross section, buccal mucosa tissue stains positive only on the luminal surface (data not shown). As documented earlier, this reflects the fact that epithelial cells contain mucin binding protein (MBP) that specifically interacts with mucin, and thus establishes mucous barrier from the luminal contents (13).
Fig. 1. Effect of ethanol on the synthesis of MBP in rat buccal mucosa. Rat buccal mucosa (1 cm²) was excised from the oral surfaces and subjected to 3 hours culture in the presence of ethanol and [³H]proline and [³H]palmitate as described in Methods. The amount of radioactivity incorporated into MBP is expressed in cpm/mg of protein. Results are means ±SD of four independent experiments with each concentration of ethanol, and under control conditions.

Fig. 2. Release of [³H]palmitate labeled MBP into the culture medium. The results are expressed in % of total MBP synthesized during the 3 hours of tissue culture performed in the presence of ethanol and radiolabeled proline and palmitate. The means ±SD are calculated from the results obtained from three samples prepared for each experiment and each concentration of ethanol.
To determine effect of ethanol on the performance of oral mucous barrier, the MBP from buccal mucosa subjected to tissue culture in the presence of ethanol was investigated. After 3h tissue culture in the presence of 0-1.5 M ethanol, the labeling of MBP with palmitate and proline was characterized. The synthesis of MBP in the presence of ethanol at concentration of 0.2, 0.5 and 1.5 M was reduced substantially (Fig. 1). On the average, in the presence of 0.2, 0.5 and 1.5 M ethanol MBP synthesis decreased by 70, 90, and 95%, respectively. However, the incorporation of ethanol into the tissue culture had no impact on the release of MBP from the membranes. Consistently, in every preparation up to 82 % of MBP was released from the membranes to the medium (Fig. 2).

The analysis of the soluble material, released to the medium revealed that the released from membrane soluble MBP was labeled with palmitate (Fig. 3), but was not partitioning to Triton-X114 phase (Fig. 4). Treatment of the medium-derived MBP with nitrous acid generated palmitate-free MBP (Fig. 5). However, the nitrous acid released palmitate labeled product, on thin layer plate migrated faster than PI or acylated PI that is hydrolyzed from GPI-linked MBP of apical membranes (Fig. 6).

![Graph showing release of [3H] labeled MBP](image)

Fig. 3. Detection of the radiolabeled [3H]palmitate and [3H]proline in MBP released to culture medium. The WGA affinity purified MBP fractions from experiments containing radiolabeled [3H] proline and [3H]palmitate were subjected to SDS-PAGE in 7% acrylamide gel. The gels were cut into 12 equal pieces, dissolved in scintillation cocktail and subjected to scintillation counting. As evident from the analyses, the area corresponding to 97 kDa MBP contained radiolabeled palmitate and proline. The amount of MBP applied to gel corresponds to 50 µg of protein of culture medium.
Fig. 4. Partition of MBP from culture medium in Triton-X114 system. The Triton-X114 phase of the culture medium MBP is applied in lanes 2,3; the water phase is in lanes 4,5. The protein was detected on western blots with Glyco-Stain. The amount applied to lanes 2,4 corresponds to 5 ng of MBP, and lanes 3,5 contain 0.5 ng of MBP. Lane 1 shows prestained high molecular weight markers from Sigma.

Fig. 5. Release of palmitate from water soluble MBP with nitrous acid hydrolysis. The palmitate labeled MBP subjected to HONO hydrolysis is shown in lane 1, the mock hydrolysis of palmitate labeled MBP with HONO$_2$ is shown in lane 2, and the proline labeled water soluble MBP isolated from parallel experiment is depicted in lane 3. The samples of culture medium (50 µg) were applied to 5% SDS-PAGE, tranblotted to cellulose and subjected to radioscanning in Berthold Linear Analyzer. Each lane in the transfer was counted for 10 min and the results are expressed in counts/ area/10 min.
The fact that the released to the medium palmitate labeled material was not extracting into Triton X-114 and the material released with HONO migrated faster than PI or acylated PI, suggested that MBP released from the cell surface was derived from phospholipase D-like activity. Since the published evidence suggests that the GPI-PLD is abundantly present in tissue, particularly in serum and plasma, and is specifically inhibited with EDTA (26-28), we performed the experiments with serum-derived GPI-PLD in the presence of the chelating agent. As shown in Fig. 7, addition of the rat serum to the apical membrane preparation containing GPI-linked MBP depleted MBP from the sample (lane 2), but in the presence of 5 mM EDTA the release of MBP from the GPI anchor was inhibited (lanes 6,7).

The experiments with the fractions obtained from the preparation of the apical membranes demonstrated that the soluble fraction of buccal mucosa homogenate

![Fig. 6. Release of palmitate from MBP soluble in the medium by HONO hydrolysis. The HONO hydrolysis products are derived from the hydrolysis of the water-soluble, palmitate-labeled MBP and the butanol extract of pronase digested MBP consisting of two palmitate-labeled glycans. From both sources, the HONO hydrolyzed samples produced material migrating just below solvent front (shown in the upper right corner of the rendered plate). The composite thin layer chromatographic plate depicting mobility of the palmitate labeled material released from the water soluble MBP and the amount of the released palmitate from glycans representing four different preparations is shown as counts/sample, the migration of the glycans and the radiolabeled material was established with orcinol stain (13). Although, not typical of the orcinol stain, the material corresponding to HONO released radiolabeled component from water soluble MBP stained yellow-pink. Further analysis of the component as described in (28) revealed the presence fatty acid methyl esters of palmitate and inositol (data not shown).](image-url)
contained the activity that contributed to the release of MBP from GPI anchor (Fig. 8, lane 5). In addition, the saliva collected from rats subjected to chronic ethanol diet caused release of MBP from the membranes (Fig. 8, lanes 2-4). In both instances, however, the addition of 5 mM EDTA inhibited release of the MBP to the medium (Fig. 8, lanes 6,7).

Both fractions of MBP, the soluble and the membrane-derived, generated complex with mucin, and in nonreducing PAGE migrated as high molecular weight product that contained radiolabeled MBP and mucin (Fig. 9). This observation suggests that short term exposure to ethanol, in contrast to chronic ethanol treatment, had no effect on
MBP-mucin complex formation, but promoted release of the complex from apical membrane. Also, it appears that the acute treatment with ethanol has not impacted the MBP glycoforms. The complete digestion of soluble MBP with pronase and extraction of the digest with butanol revealed, in ethanol-derived and in controls, the presence of two major palmitate labeled glycans (Fig. 6). The presence of the two major glycans was consistent, and appeared in all preparations of water-soluble MBP of buccal mucosa obtained from several separate preparations (four preparations are shown in Fig. 6).

**Fig. 9.** Interaction of water soluble MBP with rat salivary mucin. The gel stained with silver stain is shown in panel A, and the radioactivity scanning is depicted in panel B. The radioactivity scanning was performed with Berthold Linear Analyzer on gel transfers to nitrocellulose membrane. The lanes 1-5 in panel A correspond to palmitate labeled samples derived from tissue culture in the presence of 0, 0.05, 0.1, 0.2, 0.5 M, ethanol, respectively.
The labeling status of the medium-soluble MBP, the results of hydrolysis of membrane-bound MBP with serum-, saliva- and homogenate-derived PLD, nitrous acid hydrolysis products, and the inhibitory effect of EDTA suggest that the soluble MBP is generated by the action of GPI-PLD. The released MBP from the apical surfaces of buccal mucosa in our experiments was most likely the product of activity present in culture medium, and of serum transudate in the saliva of alcohol-fed rats. Whether the GPI-PLD activity is acquired only in case of the tissue admixed with serum elements, and serum transudation with ethanol exposure, is the subject of further investigation. The results of the study presented here provide support to the contention that buccal mucosa MBP is susceptible to GPI-PLD that is attenuated by the presence of mucin.

DISCUSSION

Since the first implication of mucin role in mucous barrier formation, conflicting models have been proposed to account for its structure, properties, and function (10,11,29). The placement of mucin insulator between the cell and the external environment, however, is still not acknowledged for its critical intent. The present interest in mucin genes generated staggering amount of details on apomucin nuances that produced merely academic knowledge without furthering insights into the functional capacity of the finished product (30-32).

In our concept, the most critical function of mucin lies in modulating and screening signals coming from lumenal environment. While the attention to the importance of modulating and screening signaling processes is considered and recognized as highly specific phenomenon, attributed to stem cells that can be transformed into range of cell types, it is the same virtue that ascertains characteristic behavior of the epithelial cells. The medium delivering extracellular signals to stem cells or to absorptive epithelia exerts its functional mark on the exposed cell. Therefore, in the epithelia of oral, pulmonary, gastrointestinal and urogenital tract, it is quintessential that the passing signals are not changing characteristics of cell or affect its receptor stability and their availability. Such a selective barrier is provided by mucin.

In our initial study on mucous barrier, we have demonstrated that the specific barrier on gastric and oral mucosa is assembled by the interaction of the secreted mucin with membrane anchored Mucin Binding Protein (MBP) (13,14). The retention of mucin on the apical epithelial surfaces is dependent on mucin glycosylation and the presence of the specific determinants on mucin oligosaccharides (12,13). Further studies revealed that in gastric mucosa without mucin occupying the binding sites on MBP, the H. pylori-derived LPS couples with the MBP (20). From the above experiments and the study with animals subjected to intragastric administration of LPS we concluded that in gastric mucosa the LPS-induced processes are occurring when mucin displacement is generated experimentally with topical application of
mucolytic agents or by metabolic intervention (21, 23). The evidence for the latter was obtained in the studies of aberrantly glycosylated mucin from rats subjected to chronic ethanol diet (12). Mucin samples collected from the individual rats over period of 9 weeks were analyzed for binding activity with its own MBP. In each set of mucin samples, the analysis revealed a drastic decrease in binding of mucin to its MBP. On the average, a 70% decrease in mucin binding to MBP and 75% reduction in mucin retention on gastric epithelial surfaces of the ethanol-fed rats was recorded, while in several rats the features of mucin binding and its retention were not measurable. Based on the results described, we postulated that mucin interaction with MBP is controlled by the posttranslational glycosylation of mucin, and that mucous barrier assembly is disturbed by the substances interfering with the process of posttranslational glycosylation.

Our initial aim in the studies with buccal mucosa was to determine whether ethanol induces similar changes in mucous barrier of buccal mucosa as in gastric epithelium by affecting the buccal MBP and thus changing effectiveness of mucous cover. Indeed, the depletion of the mucous barrier was evident in histological samples and correlated with introduction of ethanol to the primary tissue culture (33). However, analysis of the soluble components of the mucous barrier, that were recovered from the medium, suggested that all samples of buccal mucosa incubated with and without ethanol released the same, (up to 80 % of the mucous barrier) components that included mucin and de novo assembled MBP. Based on our analyses, the fact that GPI-anchored MBP was found in the medium, as water soluble component that contained palmitate label, suggested GPI-PLD-specific cleavage (25-27, 34). As in the case of buccal mucosa preparations, similar findings were reported for heart, platelets, and human placenta GPI-anchored APase (35-39). The release of membrane anchored MBP, just as the APases, was sensitive to chelators (EDTA, EGTA and thiol-blocking reagents) (35,36,40). As ascertained in number of studies, that feature is specific for GPI-PLD activity abundant in plasma and serum (25-27). Therefore, under normal conditions, when the oral tissue is intact, the MBP of apical epithelial cells remains membrane bound. However, with ethanol, detergent or 2M NaCl, as we found previously (41), serum transudates to the secretion. This would infer that serum GPI-PLD may often contribute to the release of mucous barrier from oral and gastrointestinal epithelia. After all, these surfaces are often subjected to conditions that provoke serum transudation.

On the other hand, it is also established that GPI-PLD is not acting on GPI-anchored proteins in their native membrane environment (37-39, 42). What would then trigger its activity against GPI-linked proteins? After all, alkaline phosphatase, heparan sulfate proteoglycan, lipoprotein lipase, CD16, CD14, N-CAM, oligodendrocyte-myelin protein, CEA are found in soluble and GPI-anchored form. In many cases, their levels are altered by disease and physiological stimuli, but in general, the functional aspect of the change is still not discerned. It is speculated that membrane shedding or secretion
of the soluble form may contribute to multiple forms of the same protein, and that GPI anchor does not serve any special function (38,39). Our exploration revealed that utilization of Triton X-100 to isolate apical epithelial membranes (24, 43), produced mucin-free preparations which in the presence of protease inhibitors and EDTA retained the membrane anchored MBP. These membranes, however, without EDTA in the medium released MBP in the water-soluble form. In our interpretation, this suggests that GPI-anchored protein of apical epithelial surfaces, normally engaged in the interaction with mucin, is insulated and protected from the lumenal contents. This is the insulator function we assign to mucin that interacts with MBP and protects the GPI-anchor from lumenal proteases, and serum transudates containing GPI-PLD. This speculation is supported by the findings that the MBP in rats subjected to chronic ethanol diet, that is not well protected by modified mucin was found in water soluble form (data not shown). The direct relationship between soluble MBP, lose of function to interact with MBP in mucin derived from chronic alcohol feeding, renders support to our notion that in the absence of mucin the membrane component of mucous barrier is susceptible to GPI-PLD. It is quite possible that a similar arrangement contributes to the release of the GPI-anchored proteins of other origin (34-37).

Several lines of evidence suggest that GPI-PLD might participate in regulating inflammation in atherosclerosis (44). GPI-PLD is highly specific for atherosclerotic as compared with nonatherosclerotic tissue (44). This fact is used as the evidence that GPI-PLD is involved in inflammation. Indeed, the enzyme is expressed by and colocalized with the subset of plaque macrophages. Yet, the major question remains unanswered whether the initial injury to the arterial tissue, that is associated with inflammatory response, is in first caused by the conditions that initiate degradation of GPI-anchored protein complex of the arterial wall.

Perhaps, the speculations concerning the functional significance and the role of GPI-anchored membrane protein require different view. It is reasonable to assume that GPI anchor is required to protect the cell from autodegradation which could materialize with other type of membrane anchoring. Particularly in view of the numerous proteases that are localized to membrane (45) and could easily degrade the protein in an immediate surrounding of the cell surface, or as is the case in digestive tract where the digestive enzymes are bounteous. In both instances, the role of GPI anchor is justified. At the same time, as we argued above, the GPI-protein complex may provide a specific screening system that guards cells from nonspecific and undesired signals, and prevents interference with cellular processes that establish its characteristics (46-49). The same assumption that GPI-anchored protein serves to interact with an external specific protein and thus controls and modulates extracellular signals in the arterial wall, is also plausible (44).

How does the epithelial cell with its own set of programmed functions defend itself against the external environment of the alimentary tract? Work over the last several decades suggests that mucin is responsible for providing the barrier against
the intrusions from the surrounding medium. The fact that mucin, the insulator, vary greatly reflects on its ability to act as the specific positional blocker. The primary encounters with pathogen and the capacity of the mucous complex to block its contact with the underlying cells depend on mucin glycosylation (memory), just as the first encounter with pathogen is remembered by immune system (50). Just as the naive T cells that respond to antigen by undergoing profound changes in the surface molecules that they express, the mucin producing cells reflect the encounters with pathogens in multiplicity of mucin carbohydrate determinants with unlimited ability to bind pathogen. Therefore, the real danger is not in microbe’s binding property, but in the lack of recognition and preparation to block its access to cells, and the removal of the organism.

It is well known that microbes developed numerous ways to dismantle the protective mucous barrier. A direct way of nullifying the action of an insulator has been developed by microbes that succeed in production of lipopolysaccharides that are equipped with carbohydrate determinants that mimic mucin (51,52). It appears that such decoys produced by the bacteria derail function of mucous barrier. The important issue here is to find out whether displacement of mucin from MBP by LPS signifies the final brake of the blockade, initiation of LPS-induced signaling and free access of pathogens to underlying cells. However, possibility still exists that the MBP-LPS complex then becomes susceptible to GPI-PLD and is released to the medium. If indeed this is the path to reject LPS-induced signaling, this would justify the abundance of the GPI-PLD in serum and the GPI anchor as the major posttranslational modification of apical membrane proteins. Obviously, their function would be to eliminate the microbe-induced changes.

In the concept defended here, the role of mucous barrier in preservation of normally functioning epithelia in the entire organism is comparable to epidermis, and the scope of this function is vast and yet incomprehensible. Alone, the possibilities that with proper attention to housekeeping of the epithelial barrier that separate our organism from environmental agents and microbial kingdom, cancer and infectious diseases could be prevented or controlled, is staggering. Our ingenuity must concentrate on preparing the entire organism to face the challenges of changing surrounding and not be limited to skin only.

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