A. SLOMIANY, E. PIOTROWSKI, J. PIOTROWSKI, B. L. SLOMIANY

IMPACT OF ETHANOL ON INNATE PROTECTION OF GASTRIC MUCOSAL EPITHELIAL SURFACES AND THE RISK OF INJURY

Research Center, University of Medicine and Dentistry of New Jersey, Newark, USA

Earlier investigations on the effect of ethanol on synthesis and posttranslational glycosylation of gastric mucus glycoprotein (mucin) revealed quantitative changes in the apoprotein assembly, glycosylation, and mucin retention on the mucosal surface (Sloomiany et al., Alcoholism: Clin. Exp. Res. 21, 417–423, 1998). To assess whether metabolic consequences of ethanol ingestion, documented in the in vitro system are also occurring in vivo the rats were subjected to 8 weeks of ethanol containing liquid diet. The retention of mucin on the surface of gastric mucosa was quantitated by measuring the binding of gastric mucin to Mucin Binding Protein (MBP) of gastric mucosa. The results were compared with those obtained with the rats subjected to pair-feeding the isocaloric-control diet. Before alcohol administration, and in two weeks' intervals thereafter, the gastric contents from the animals was collected and mucin purified. After 8 weeks of the respective diet, the animals were sacrificed and their gastric mucosa used for MBP preparation. The binding of mucin to MBP before ethanol, and after 2, 4, 6, and 8 weeks of ethanol diet was quantitated with Enzyme Linked Lectin Assay (ELLA). The study with standard mucin revealed that binding of mucin to MBP differs substantially between individual animals. The same variability in binding was observed with the individual mucin preparations collected at the onset of the experiment. However, with the progression of ethanol feeding, the mucin samples besides displaying the variable and animal-specific binding to MBP at the initiation of the experiment, also showed a dramatic decrease in binding. In five animals, after two weeks of ethanol diet, mucin binding to MBP decreased by 50%; in two animals, the drastic decrease in binding was observed in mucin collected after four weeks of alcohol feeding; and in one animal a 20% decrease in binding persisted for six weeks, and then decreased to 50% in the last collection. Also, in two animals, the mucin collected after 8 weeks of ethanol feeding retained only 6–9% of the initial binding capacity. In contrast, in pair-fed controls, the mucin binding to MBP remained the same or increased up to 20%. Results of the studies, performed on mucin of the individual animals and matching preparations of MBP, showed that each animal expresses different degree of mucin binding. Moreover, in chronic ethanol ingestion, the individual variations are accompanied by a decrease in mucin binding to MBP. Since the observed decrease in binding occurred in samples containing the same preparation of MBP, the component affected by alcohol resides on mucin. Thus, considering the in vitro impact of ethanol on generation of carbohydrate chains in Golgi, and the finding on mucin oligosaccharides-dependent mucin-MBP complex formation, we conclude that ethanol impairs the synthesis of mucin oligosaccharide structures required for binding with MBP, and the retention on gastric mucosal surfaces.

Key Words: ethanol, innate protection, gastric injury, mucin glycosylation, mucin binding protein.
INTRODUCTION

The protective mucous gel produced by gastrointestinal, oral, pulmonary and urogenital epithelia, and the epidermis, the outer covering skin generating keratinized squames, are rarely compared. Yet, just as skin with its keratinized zone, the epithelia with their mucous layer are irreplaceable in the protection of the underneath cells from mechanical, chemical and microbial injury (1—3). Mucus glycoproteins, the molecules that impart protective character of the epithelial zone, although investigated for several decades, are not adequately recognized as highly relevant and specific impediment for microbes (4—14). It is still difficult to particularize the significance of multiplicity of mucin carbohydrate chains, or to verify the impact of diet and carbohydrate metabolism on the extremely complex and deceptively unruly posttranslational glycosylation.

In our laboratory, the significance of mucin glycosylation has been brought to the forefront by the finding that the adherence of mucin to gastric epithelium is controlled by the carbohydrate-specific interaction between mucin and the epithelial mucin binding protein (MBP) (13, 14), and that in the absence of mucin, the H. pylori lipopolysaccharide can bind to MBP and engage farther signals (13). Therefore, the task in this study was to establish whether the consequences of chronic ingestion of ethanol reflected in mucin synthesis have the impact on binding of mucin to epithelial MBP. Considering the facts that in vitro and tissue culture environment are not imitating exactly the in situ glycosylation processes (15—17), the study required the development of an experimental design where the individual animals exposed to ethanol could be investigated.

In this report, we present quantitative results on mucin binding to its epithelial MBP, conducted with rats subjected to 8 weeks of chronic ethanol feeding. The nature of changes assessed separately on the individual animals, supports the earlier findings that ethanol affects gastric mucous barrier formation by curtailing mucin binding to the epithelial MBP.

METHODS

Animals

The animals were fed liquid diet containing ethanol (6.7%v/v ethanol; 37% ethanol derived calories) or control liquid diet containing carbohydrate caloric replacement in form of maltose and dextrin. The premixed diets, formulated as described in (18), were purchased from Bio-Serv, Freington, NJ. Sprague-Dawley rats weighing 110—130 g were immediately introduced to liquid-diet, and after 3 days divided into two groups where one was receiving the same liquid diet, and the other half was gradually introduced to ethanol-liquid diet by mixing 1/3 of ethanol liquid diet with 2/3 of control. After 3 days, the ethanol-fed group obtained 2/3 of ethanol containing diet and 1/3 of control. Three days later, the animals were weighed, matched in weight with rat on control liquid diet and placed in the individual metabolic cages. The ethanol-fed rats were then introduced to a full ethanol-containing liquid diet and each day the amount consumed was
measured so that the pair-fed control would receive the same volume of control diet on the following day. The pair feeding experiment was continued for 8 weeks.

**Gastric mucin collection**

Gastric mucin was collected from each rat at the initiation of pair feeding experiment and after 2, 4, 6 and 8 weeks. The night before collection, the liquid diet was withheld, and in the morning 2–4 ml of saline was introduced to the stomach of each animal. After 15 min., the liquid was collected by introducing narrow tube to the stomach and removing the contents. Thus obtained stomach contents were immediately neutralized, treated with DNase (19) and protease inhibitor cocktail (Sigma), centrifuged and dialyzed. Thus prepared samples were used for isolation of mucin (20). The purified mucin was quantitated using peroxidase labeled WGA lectin (21). The assay gives quantification of the number of specific binding sites that are available in the given glycoprotein for the specific reaction with labeled lectin. Thus, it is particularly useful for obtaining the information on mucin derived from the same animal.

**Enzyme Linked Lectin Binding Assay (ELLA)**

Aliquots of 100μl mucin from 1ml total volume, reconstituted from gastric contents collections described above, were applied to the ELISA plates (Dynatech) and diluted out with 100 μl of carbonate buffer, pH 9.5 (21). After 16 h at 4°C, the mucin aliquots were removed, the wells washed twice with phosphate buffered saline containing Tween 20 (1 ml/l) (PBS/Tween), and incubated with 120 μl blocking buffer consisting of PBS/Tween and 0.2% bovine serum albumin for 4h at 37°C. The blocking buffer was removed, the wells washed three times with PBS/Tween and 100 μl of peroxidase labeled-WGA lectin (2μg/ml in PBS/Tween) was introduced and incubated for 4h at 37°C. The lectin solution was then removed, the wells washed five times with PBS/Tween and developed with 100 μl orthophenylendiamine (OPD) substrate (10 mg tablet (Sigma) dissolved in 25 ml phosphate/citrate buffer, pH 5.0, to which 10 μl 30% hydrogen peroxide was added). After 6 min. incubation with the substrate, 100 μl of 4 M sulfuric acid was added and the optical density at 492 nm was determined. The plates were read after 30 min. at room temperature and again after overnight at 4°C. To account for the variation in binding of the individual plates, each plate was run with mucin standard, prepared and aliquoted at the beginning of the experiments. The dilutions of standard rat gastric mucin were made from the stock at concentration of 1mg/ml. The standard mucin at concentration 1.25 μg/ml, in binding assay with peroxidase labeled lectin at 492 nm, was equivalent to one absorbance unit (IA492). Each measurement was performed in triplicate, and repeated with each set of samples obtained from the individual animals.

**Preparation of gastric mucin binding protein (MBP)**

Gastric mucosal cell membranes used for MBP isolation were prepared according to procedure described (14). After 9 weeks of pair-feeding experiment, the MBP was isolated from the mucosa of individual rat stomachs and used in binding experiments with mucin collected from the same animal. After final collection of gastric contents, the animal was killed, its stomach excised, washed with ice-cold PBS, and gently scraped with soft rubber scraper to remove adherent mucus. Mucosal cells were collected and used for preparation of MBP (14). The eluates from WGA affinity columns (14) were monitored by SDS-PAGE, and the fractions containing nearly pure 97 kDa MBP were combined. For ELLA assay, the collected 97 kDa MBP was quantified, and applied at concentration of 1A492/100μl/well of ELISA plates. The average binding of the MBP to well was from 35—45%.

For the quantitation of mucin binding to MBP, the ELISA wells coated with MBP were washed and blocked; reacted with mucin at concentration of 1A492/100μl, washed and blocked again; reacted with peroxidase-WGA lectin, washed and developed with OPD substrate (21, 22).
To minimize variations, the standard gastric mucin, the mucin collected from ethanol-fed, and the pair-fed control, were quantified at the same time and on the same plate. Each measurement was performed in triplicate and repeated twice.

RESULTS

The data were acquired from the studies of 15 pairs of rats that were pair-fed chronic ethanol and control liquid diet. The pairs were established at the end of the introductory week based on the weight of the rats, and as such they remained for the following eight weeks. At the termination of the experiment, the gastric mucosa of the animals was not ulcerated.

The MBP was isolated from gastric mucosa by employing affinity column technique described earlier (14). Following quantitation at several dilutions, the receptor protein concentration was adjusted to $1A_{492}/100\mu l$ and as such used in all experiments in mucin binding.

The preparations of mucin obtained from the collections of gastric contents of the pair-fed rats on ethanol- and control-liquid diet (Table 1) were sufficient to study the mucin interaction with MBP in the ELLA system (21—23). In all quantitations involving individual collection of gastric mucin, the standard mucin was also adjusted to $1A_{492}/100\mu l$ and that corresponded to 0.125$\mu g$ mucin/well. The described conditions were applied to all sets of samples.

Table 1. Recovery of mucin from gastric aspirates of individual rats subjected to chronic alcohol (A) and control (C) liquid diet.

<table>
<thead>
<tr>
<th>Collection Pair No.</th>
<th>Mucin $\mu g/ml$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
</tr>
<tr>
<td>1 A</td>
<td>60</td>
</tr>
<tr>
<td>1 C</td>
<td>380</td>
</tr>
<tr>
<td>2 A</td>
<td>200</td>
</tr>
<tr>
<td>2 C</td>
<td>60</td>
</tr>
<tr>
<td>3 A</td>
<td>30</td>
</tr>
<tr>
<td>3 C</td>
<td>300</td>
</tr>
<tr>
<td>4 A</td>
<td>40</td>
</tr>
<tr>
<td>4 C</td>
<td>140</td>
</tr>
<tr>
<td>5 A</td>
<td>210</td>
</tr>
<tr>
<td>5 C</td>
<td>20</td>
</tr>
<tr>
<td>6 A</td>
<td>30</td>
</tr>
<tr>
<td>6 C</td>
<td>30</td>
</tr>
<tr>
<td>7 A</td>
<td>60</td>
</tr>
<tr>
<td>7 C</td>
<td>520</td>
</tr>
<tr>
<td>8 A</td>
<td>230</td>
</tr>
<tr>
<td>8 C</td>
<td>650</td>
</tr>
</tbody>
</table>
The quantity of mucin recovered from individual animals varied substantially (Table 1). Most of the variation resulted from the difficulties with recovery of the entire volume of buffered saline introduced to the stomach. Some animals were calm and easy to control, while other were fearful and difficult to keep in fixed position to collect the stomach contents. Hence, recovery of the infusates varied from 1.5 to 3.5 ml. However, as in the entire experimental set-up, the important goal was to collect the samples of mucus containing secretion without injuring or stressing the animal.

With the material collected from the four pair-fed pairs we established that incubation of MBP with mucin in the ratio of 1A_{492} 1:1 resulted in the saturation of MBP mucin binding sites. The increasing amount of standard mucin (1, 2, 4 A_{492}) did not augment further binding to MBP. Thus, it was concluded that the amount of standard mucin equivalent to one absorbance unit was sufficient to saturate binding sites of MBP. The variations in the readings obtained with multiple plates were eliminated by running the standard mucin binding and the standard binding curve for every plate. In each set of the measurements, the reactivity of individual components with WGA included: standard mucin in dilution range from 1:1 to 1:300, MBP, the mucin isolated from the stomach contents harvested during the experiment, and the blank. Each sample was measured three times, each time the reactions were performed in triplicate, and the parallel rows on the plate were used for the reaction of the receptor with mucin from pair-fed control. In this arrangement, the experimental error of the readings ranged from 6—8%.

The binding of standard mucin to MBP was not identical in all rats (Fig. 1, 2). The variation in the degree of standard mucin binding to the individual MBP preparations was also observed with mucin collected at the beginning of the pair feeding experiments (Fig. 1, 2). The correlation between the binding of standard mucin and the individual mucin preparations matching the source of MBP showed excellent relationship of 0.94—0.95.

During the pair feeding experiment, in five pair-fed control animals, the binding of mucin to its MBP increased by up to 20%, and in three remained the same (Fig. 3). In contrast, none of the mucin preparation from ethanol-fed animals exhibited unchanged or elevated binding, and the changes in mucin affinity with its MBP have not occurred at the same rate (Fig. 4). In ethanol-fed rats, from pair 1, 2, 3, 6 and 8, the drastic change in binding resulted after two weeks of ethanol feeding. In the animal from pair 5, the reduction in mucin-MBP binding was observed after 4 weeks, and in the animal from pairs 4 and 7, the effect of ethanol was detected after 8 weeks.. By the end of the experiment, in all ethanol-fed animals the mucin binding to the own MBP decreased by 50% or more, and in two animals from pair 3 and 8, the mucin was practically not binding to its MBP (Fig. 5).
Fig. 1. Binding of standard rat gastric mucin to MBP, and the mucin and the MBP isolated from the same experimental animal. The standard mucin was isolated from combined mucous gel scraped from gastric mucosa of 10 rats. The experimental mucin was isolated from gastric contents collected at the initial week of feeding the control liquid diet. The MBP was isolated from the animals after completion of the experiment. The protocol of ELLA assay is described in details in Methods. The open bars represent the results of binding of standard rat gastric mucin (Std RGM), and filled bars represent binding of mucin isolated from the same animal as MBP. The RGM was collected during the first week of pair-fed control liquid diet (pair-fed RGM). Each bar represents the mean±SD of 3 determinations performed in triplicate. The correlation factor between the values obtained for binding of standard mucin (Std RGM) and the mucin samples collected at the onset of the experiment from the individual animals representing the pair-fed controls (pair-fed RGM) is equal to 0.95.

The statistical evaluation of binding between MBP and mucin collected at various stages of the experiment depicted in the Fig. 3, reflected correlation of 0.86 and that for Fig. 4, was equal to 0.84, suggesting that variable binding is highly related in both groups to another variable, the mucin, that each time is derived from different animal. The data in Fig. 5 demonstrate the percentage change in binding in alcohol-fed, and pair-fed controls. Each point relates to the initial MBP-mucin binding held as 100%, whereas the corresponding values reflect the binding values obtained with mucin collected after 8 weeks of the respective diet. Considering that MBP used in the experiments depicted in Fig. 3 and 4, was the same as in studies presented in Fig. 1 and 2 and derived from individual animal, the results demonstrate that ethanol changed mucin ability to bind to MBP.
Fig. 2. Binding of standard rat gastric mucin with MBP, and the mucin and MBP isolated from the same animal. The MBP in both studies is derived from gastric mucosa of the individual animals (alcohol-fed animal 1—8) after 8 weeks of alcohol diet. The standard mucin was the same as in the studies depicted in Fig. 1. The experimental mucin was isolated from gastric contents of the rats chosen for the pair feeding of the chronic alcohol diet before ethanol containing diet, and the MBP was isolated from gastric mucosa of the same animals after the completion of 8 weeks of chronic alcohol diet. The open bars represent the binding of standard rat gastric mucin (Std RGM) to the MBP, and the filled bars depict the results of binding between mucin and MBP derived from the same animal. The protocol of ELLA assay is described in Methods. Each bar represents the means ± SD from 3 determinations performed in triplicate. The correlation factor between the binding of standard rat gastric mucin (Std RGM) and the mucin samples collected from individual animals before subjecting them to alcohol diet (Alcohol RGM) is equal to 0.95.

In previous study (14) we demonstrated that the binding of mucin to MBP is dependent on the presence of mucin oligosaccharides. Hence, the chronic ethanol-induced changes in binding are interpreted as ethanol-abated synthesis of the carbohydrate epitopes that are necessary for the MBP-mucin binding. From the preliminary studies with control mucin (unpublished results), we have determined that MBP binds oligosaccharides exhibiting thin layer chromatographic migration corresponding to that of octa-, deca- or larger oligosaccharides. Thus, it appears that chronic and acute ethanol consumption, by affecting elongation of mucin carbohydrate chains, contributes to the depletion of mucin from epithelial surfaces that leads to inevitable damage of mucosal epithelia. As to the observed differences in the initial binding of mucin to its MBP, it is conceivable that the inherent poorer binding of mucin might be the major factor that predisposes gastric mucosa to injury.
Fig. 3. Binding of mucin derived from the animals subjected to the pair-fed control liquid diet collected at the beginning of the experiment (W0) and after 2, 4, 6 8 weeks (W2, W4, W6, W8) to MBP isolated from the same animal after the last collection of gastric contents. The protocol for ELLA assay was as described in Methods. In all figures the results refer to the same pairs of pair-fed animals. Each bar represents the means ± SD from 3 determinations performed in triplicate. The correlation factor between sets of data for week 0 (W0) and week 2, 4, 6 and 8 is 0.86.

Fig. 4. Binding of mucin derived from the animals subjected to the pair-fed chronic alcohol liquid diet to MBP isolated from the same animal after the last collection of gastric contents. The experiment was performed exactly alike the one described in Fig. 3. The mucin samples were prepared from gastric content of rats on ethanol-liquid diet collected in two weeks interval (W0, W2, W4, W6, W8). The binding of mucin with its receptor was determined by ELLA assay described in Methods. Each bar represent the means ± SE of 3 determinations performed in triplicate. The correlation factor between the set of data obtained for week 0 (W0) and week 2, 4, 6, and 8 is 0.84.
Fig. 5. The change in binding of mucin to MBP in animals subjected to 8 weeks of chronic alcohol diet and in corresponding pair-fed controls. In each group, the initial binding of mucin to its receptor is depicted as 100% (W0). The results of mucin binding to MBP after 8 weeks of control liquid diet (W8C) and chronic alcohol liquid diet (W8A) are depicted as shown in legend. The numbers on horizontal axis represent the same pair-fed pairs as shown in all figures and in the Table 1. Each point represent the mean percentage of binding compared to the initial binding at the inception of the experiment.

DISCUSSION

The stripping of mucous barrier from epithelial surfaces of oral, gastrointestinal, pulmonary or urogenital tract is not easily detected and treated. The gastrointestinal open wounds, the ulcers, if not excessively bleeding, remain unprotected from microbes, toxins and acid injury. The fundamental question here is why during the decades of investigation on innate protection and immunity, the basic function of mucin was not recognized adequately and most of the antibacterial protective functions were delegated and attributed to immuno response (24—27). Presumably, the low regard for the function of mucin as pathogen binding agent resulted from the lack of common knowledge how vulnerable to bacterial and viral assault, colonization, invasion, and toxins are the epithelial cells without the mucin protection (13, 28—31). We have settled for the most obvious function of the mucus
glycoproteins as the lubricants of GI tract, and very little if any research on the role of carbohydrate in fending epithelia has been continued (8, 9, 16, 17). There are no studies showing whether the different mucin genes secure for the epithelium the advantage of protection, or whether the function is independently procured from posttranslational modification (32, 33).

In the study presented here, we supply evidence that mucin binds to the membrane-anchored MBP and that its retention diminishes in rats subjected to feeding chronic ethanol diet. This reduction of the retention is reflecting the qualitative changes induced in mucin, since each time the same amount of mucin was used in binding and the same MBP used in all measurements performed on the samples of the given animal. The application of the isocaloric liquid diet, administered to pair-fed animals, provided control for each ethanol-fed animal and eliminated variations introduced by the diet. Thus, the impact of ethanol on mucin and binding the MBP was clearly established.

The results of the analysis of standard mucin binding to MBP of individual animals showed that binding was consistent, but varied within the animals. In some, mucin binding was prominent and approached 50—60% of the mucin introduced to wells, but in other remained as low as 15—20%. Since the mixed standard mucin was not increasing the binding, we interpreted this as the individual characteristic or incompetence of mucin-MBP complex. The extreme values of standard mucin binding associated with extreme binding of the mucin specific for the individual animal depicted in Fig. 1, 2 predicted high degree of correlation. The value of this analysis is that it offers some justification why some animals develop ulceration, or are affected early into alcohol treatment, whereas other displayed ability to remain healthy for the prolonged period of ethanol feeding. Furthermore, it also provides an additional interpretation as to why H. pylori attaches to epithelia of some animals or individuals while it remains harmless in others (1—3). Similarly, the binding sites of lipopolysaccharide (13) and the extent of penetration of bacterial toxins from the gastrointestinal tract would also differ in the individual animals. Inadvertently, the significant individual characteristics of gastric mucosa are camouflaged in pooled samples. The different magnitude of the mucosal protection and of ethanol-induced changes that occur in the individual animals at different time frame, in the final analysis of combined samples may appear similar in all groups and, the minor differences are attributed to the experimental error (24). Our studies clearly demonstrate, that even under standardized control conditions some of the animals appear to be predisposed to mucous barrier failure as the MBP-mucin complex is not as thoroughly protecting the epithelium as in others.

The reason for the increase in mucin binding to the MBP with the duration of the experiment in the pair-fed controls is not obvious. Under the in situ conditions, it could suggest that liquid diet is favoring slower turnover of the
mucin covering the epithelial surfaces. This, however, does not apply to the reaction in ELLA between MBP and the mucin. The only conceivable explanation would be that carbohydrate replacement (caloric equivalent of ethanol) in control liquid diet affects mucin glycosylation. If such possibility is indeed likely, this would be a first direct evidence to support the common belief regarding the nutritional value of complex carbohydrate in prevention of gastrointestinal disease. Would this proceed through the fortification of mucin synthesis and arming the mucosal barrier to fend off variety of noxious agents, including oncogenic substances?

This speculation is extremely tempting, since for any process to be initiated, the toxin, virus, or bacteria must gain access to the epithelium (34–37). If mucins are generated with the full capacity to block such an interaction, the disease causing agents stand minimal chance to bind, colonize or penetrate the epithelium. Although at this stage the idea is only speculative, it would certainly serve a good purpose if more information was available about effects of diet on mucin synthesis in human and fortification of the structures that protect epithelia from the oncogenic substances, toxins and microbial invasions (28, 31, 34–37). The argument to support such a notion is the suggested link between H. pylori and gastric ulcer and cancer (28–30, 38). Is it then possible, that a common denominator in both is the presence of unprotected-naked gastric epithelia?

At this stage, as we pointed out, there is little direct evidence that suggests the relation between the diet and the expression of mucin oligosaccharides. However, it is known that in vitro conditions generate different mucin oligosaccharides than in vivo (16). Following our notion, it may be suggested that this is the effect of different nutrients provided in culture medium as oppose to those available in the animal’s diet. The individual variations in characteristic of mucin oligosaccharides occur even in situ (7, 8). Hence, both examples seem to support our findings on the individual-specific character of mucin in the animals, and the possibility that nutrients affect the synthesis and the expression of apomucin and its oligosaccharides in human gastric mucosa.

From the analysis of the sets of data, the correlation coefficient of 0.84 suggests that MBP binding and the mucin quality are highly related and thus it allows us to speculate that mucin synthesized in the presence of ethanol is depleted of the oligosaccharides that contain specific determinants responsible for the mucin-MBP interaction. Again, the changes are of different magnitude and intensify at the different intervals of ethanol consumption. In some of the animals the effect of ethanol progressively intensifies with the time of ethanol feeding, in other, the moderate response is immediate and remains constant for up to 4 weeks and than occurs again. Also, in some animals, ethanol feeding entirely depletes mucin ability to react with MBP.
The gastric pathology in alcoholism is generally attributed to the solvent stripping effect of alcohol resulting in the gastrointestinal ulceration and bleeding (38—41). The deeper understanding of the causes of increased infections in alcoholics is attributed mainly to the research on the immuno-deficient population of HIV-positive individuals (25—27). It has been realized that bacterial pneumonia, septicemia, and other clinically observed increases in infectious diseases, transferred through mucosal surfaces, are many times more frequent in alcoholics than in nonalcoholics (27, 38). These facts are presently interpreted as alcohol induced damage to the immune system (25—27). Although, indisputably, this is also the case, primarily it illustrates ethanol-induced effects on another defense mechanism, as the mucosal immune response is triggered when the infectious agents find the way to penetrate or attach and colonize the tissue (12). The results of studies showing in pneumonia, or urinary tract infection an increase of monocytes and neutrophils recognize the secondary responses. The first line of defense against invading bacteria, that is attributed to innate immunity, reflected in mucin ability to cover epithelium and bind invading microbes, is not considered. In our interpretation, the medical complications in alcohol abuse reflected in the increased susceptibility to infection have common biochemical ground (15, 42, 43) and stem from the ethanol-induced aberrant glycosylation of surface active molecules that are incapable to interact with MBP and generate the protective cover for gastrointestinal epithelium.

Indeed, understanding the mechanism of the epithelial protection in animals may provide clues for establishing preventive measures and fortification of the first line of defense in human before the immune system is excessively burdened. Thus, to prevent the mucosal infection, the drugs that enhance the action of the incompetent mucin (44—46) and amplify the protective abilities of the mucous coat are highly desirable.

REFERENCES


Received: December 15, 1999
Accepted: July 6, 2000

Author's address: Amalia Slomiany, Research Center, C-873 University of Medicine and Dentistry of New Jersey- New Jersey Dental School, University Heights, 110 Bergen Street, Newark, NJ 07103-2400
Fax: 973-972-7020; e-mail: slomiaam @umdnj.edu