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Glycidic components expressed in the intestinal epithelium of teratomas are similar to those presented in both tumoral and inflammatory intestinal disease

Amor Ferrando, Francisco J. Fernández-Trujillo, José A. Prada, Cristina Verástegui and M.ª Carmen Martínez

Department of Morphological Sciences, Faculty of Medicine, University of Cádiz, 11003 Cádiz, Spain

SUMMARY

In this work we used several different lectins to determine the glycidic components that appear in the prismatic intestinal epithelium of teratomas along the development. These tumoral masses show structures derived from the three blastodermic layers and usually present transdifferentiative processes. Many authors have reported glycoproteic changes in malignant processes of the colonic epithelium, attending to many environmental influences, but the development of this kind of tumour does not appear to be influenced by these factors. The present data show that many of the glycidic components expressed in the prismatic intestinal epithelium of teratomas are very similar to those observed in this mucosa in both tumoral and inflammatory disease.

Key Words: Human – Glycoproteins – Differentiation – Histochemistry – Lectins

INTRODUCTION

Teratomas are tumoral masses in which the three blastodermic layers are present (Ahlfeld, 1875). Their structures are composed of a different and anarchic pattern of mature and immature tissues; they even can have organoid structures. Thus, teratomas offer a very useful material for the sequential observation of the maturational progression of the derivative tissues (Nicholson, 1929, 1930, 1934). Needham (1942) established the importance of teratomas as a tool that permits the determination of the control processes occurring in the embryonic development. Teratoma tissues, like transdifferentiative process models, appear to originate from the visceral endoderm derived from the yolk sac. Loss of the chemical brake would mean that these cells could recover their capacity of gene expression (Gardner, 1993). In teratomas it is also possible to find undifferentiated tissue parts that could afford biochemical information about cytoplasmic membrane components in this genetic deregulation.

Such biochemical changes could be attributed to the glycidic components of membrane glycoproteins, which in many cases have been implicated in several cellular adhesion mechanisms. Some authors have proposed that the malignant morphological modifications would necessarily be preceded by biochemical changes (Moore et al., 1988). It is likely, however, that multiple exogenous factors control the events that lead to these tissue alterations. In this sense, the findings of the present study could provide information about the changes that appear in such histological specimens, which have lost their mechanism of control but have not, however, received environmental influences. It is thus possible to observe cellular modifications unrelated to environmental factors, but correlated with the cellular genetic potential to express membrane glycoproteins.

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Correspondence to:
Dr. José A. Prada Oliveira. Dpto. de Ciencias Morfológicas, Facultad de Medicina, Plaza Fragela s/n, 11003 Cádiz, Spain.
Phone: 34 56 01 51 93; Fax 34 56 01 52 54; E-mail: grupo.diferenciacion@uca.es

The aim of this work is to study the intestinal epithelium. This epithelium can be seen throughout the mucosa of the digestive tract, from the cardia till the anus. Its cells are cylindrical and the nuclei are located at the same level. These cells display structural differences at the apical surface along the digestive tract, according to their function. The digestive tract derives from the visceral endoderm that appears on approximately the eighth day of gestation. This layer organises the entoblastic tube or primitive intestine.

Several authors have reported several studies about the biochemical adjustment of the sugar components in the cytoplasmic membranes of simple prismatic epithelial cells, both under normal conditions (Bresalier et al., 1985; Jass et al., 1994; Brinck et al., 1995; Falk et al., 1995) and in various pathological processes. These reports afforded important insight into the biochemical changes related to early differentiative changes in both inflammatory and tumoral disease (Hakomori, 1985; Moore et al., 1988; Chen, 1989; Pittschieler et al., 1994; Rameshkumar et al., 1994; Alessandri et al., 1995; Sharma and Schumacher, 1995).

In this study, using multiple lectins we determined most of the possible glycidic components of cytoplasmic membranes. Lectins are vegetable or animal proteins or glycoproteins that are able to agglutinate different sugars of high specificity (Goldstein and Hayes, 1978). For this reason, they have been used as a valuable tool to gain information about the probable existence of a specific sugar at their precise cellular location (Sharon and Lis, 1989). Some endogenous lectins have been implicated in specific cellular mechanisms. An example is Galectin-3, selective for terminal galactose residues, which increases with dysplastic or metastasic processes at colon level (Schoeppner et al., 1995).

The purpose of this study was to gain insight into temporal evolutionary changes, differentiative or atypical, that occur in the glycidic components of the simple prismatic epithelium. This should afford knowledge of the biochemical basis in pathological human processes at the level of such sugar residues. In this case, these changes arise from a tissue not subject to the influence of exogenous environmental factors, but with a strong tendency to show transdifferentiation and tumoral development. We believe that the existence of changes in cellular glycidic components resembles the dynamic and evolutionary processes of the intestinal epithelium would probably be due to dysregulatory changes and not to exogenous influences.

Materials and Methods

In this work we used tissues from eighty surgical samples of human teratomas, obtained at the Pathology Department of the Faculty of Medicine of Cádiz. In these samples we found types of epithelia at several stages of differentiation. The samples were fixed in Bouin for 4-6 hours and dehydrated in graded alcohol. Then, the pieces were cleared in xylene and embedded in semisynthetic paraffin (Histowax, melting point 57°C). The samples were processed routinely and 5 µm thick sections were obtained. The sections were kept in a moist chamber at 37°C for 20-30 min. Then, paraffin was removed with three steps in xylene for 5 min each, one step in a 1:1 mix of xylene-alcohol; continuous steps through degraded alcohols and distilled water for 5 min. The staining for morphological overview was performed with a modified haematoxylin of Harris-VOF of Gutiérrez (Gutiérrez et al., 1963).

The battery of lectins employed is shown in Table 1, and was as follows: ConA-HRP (Sigma L-6397), DBA-HRP (Sigma L-4258), RCA I-HRP (Sigma L-2633), SBA-HRP (KEM-EN-TEC L-2400), WGA-HRP (Sigma L-3892), UEA I-HRP (Sigma L-8196), VVA-HRP (Sigma L-5641), ECA-HRP (Sigma L-9015), PNA-HRP (Sigma L-7759) and PHAP-HRP (Sigma L-9017). Controls were made with tissues known to stain positive. For negative controls, the samples were incubated with the specific hapten sugar for each lectin at a concentration of 0.2M (see Table 1).

The sections were deparaffinized in xylene and rehydrated in degraded alcohol series and distilled water. Endogenous peroxidase and pseudo-peroxidase activities were inhibited by treatment in 3% H_2O_2 solution for 30 min. Sections were washed twice in 0,1 M, pH=7.4, phosphate buffered saline (PBS, Sigma P-4417) for 5 min and incubated in lectins in a concentration of 20 µgr/ml of PBS for 72 hours, washed twice in PBS for 5 minutes. For colour development, a solution of 10mg/15ml diaminobenzidine (3,3' diaminobenzidine, Sigma D-5905) in Phosphate Buffer Saline (Sigma T-5030) was employed in the presence of 20 µl/10ml H_2O_2, for 10 min. Afterwards, sections were dehydrated and coversliped with Entellan (Merck, 7961).

Con A had been incubated with Ca^{++} and Mn^{++} cations in a concentration of 1 mg/ml.

Table 1.- Lectins used and their specific hapten.

Lectins	Abbreviation	Specific sugar
Canavalia ensiformis	Con A	αman>αglu>GlcNac
Ricinus communis	RCA-I	βgal>αGal>>GalNac
Dolichos biflorus	DBA	GalNacα1,3GalNac
Triticum vulgaris	WGA	GlcNac > Neu5Ac
Glycine maximus	SBA	α and β GalNAc
Ulex europaeus	UEA-I	α L-fuc
Arachis hypogaea	PNA	Galβ1,3GalNac
Vicia villosa	VVA	GalNacα1,3Gal
Erythrina hipogaea	EGA	D-Gal
Phaseolus vulgaris	PHA-P	Oligosacharidics

RESULTS

In most of the teratomas we found epithelial structures with enteric differentiation and even with organoid structures akin to the vermiform appendix. The different techniques with lectins revealed several different reactive patterns. No staining was seen in sections exposed either to substrate without lectin or to lectin preincubated with the specific hapten. Meanwhile in positive control tissues the staining was intense.

Reactivity with Con-A was absent in the superficial epithelium, although mannose-glucose residues were isolated in some zones in the supranuclear cytoplasm and a discrete degree of was seen in the apical edge of related epithelium. The glan-

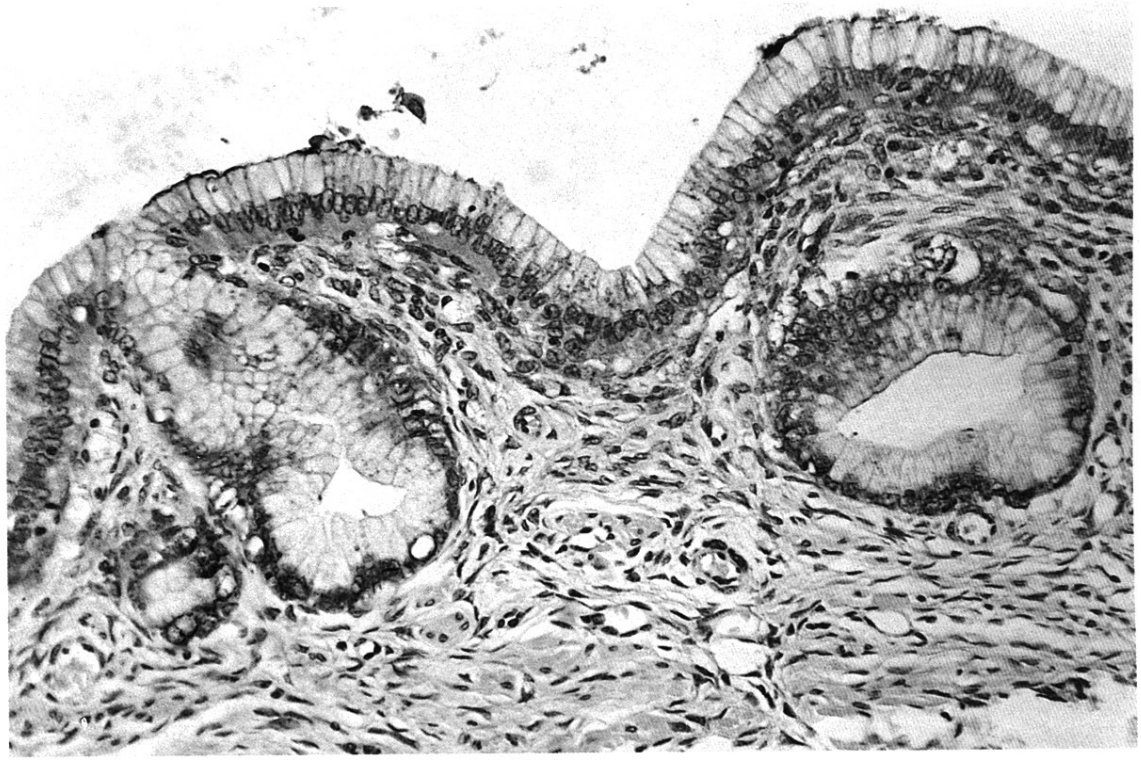

Fig. 1.- Con-A-HRP. Glandular crypts were positive for this lectin, whose binding was more intense at glandular fundus. x250.

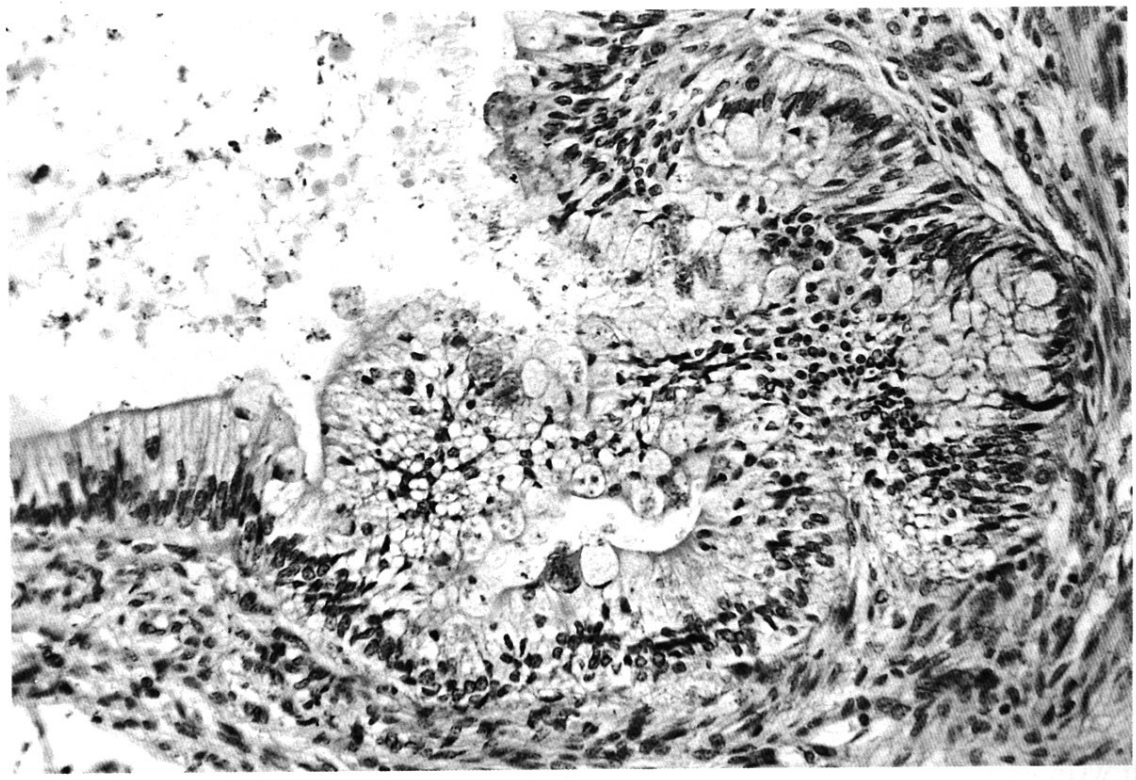

Fig. 2.- DBA lectin. Isolated cells at level of the epithelium and the glandular crypt. x250.

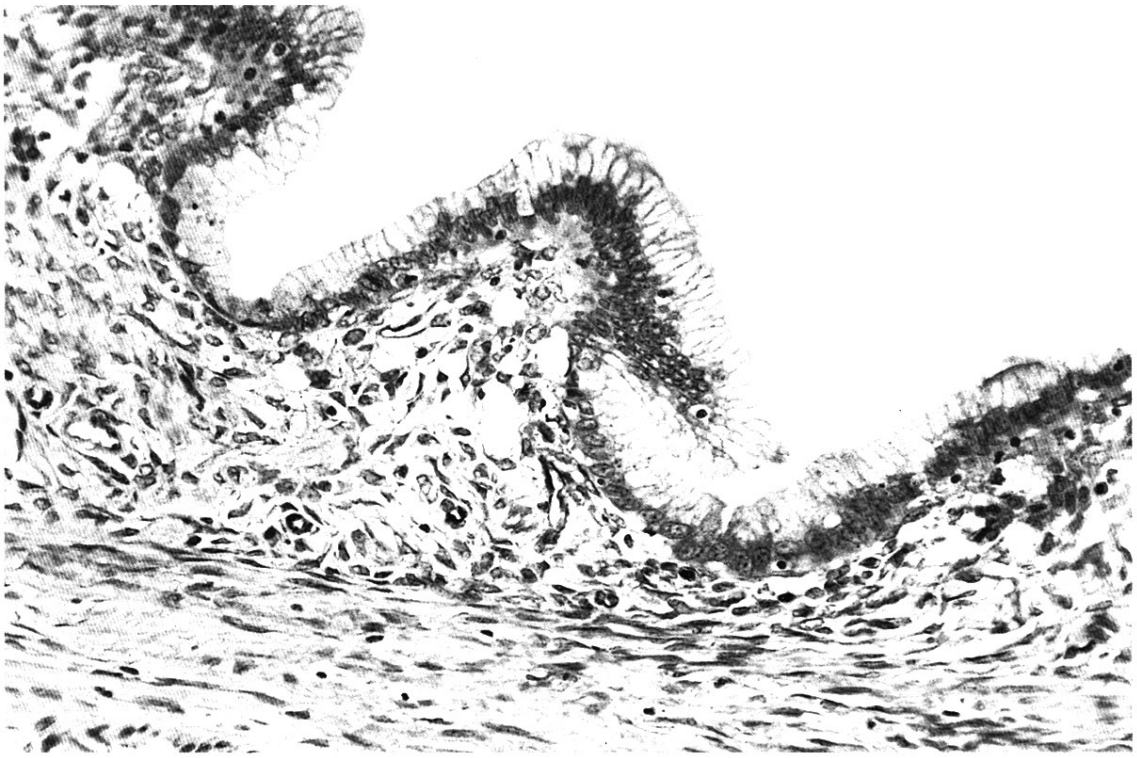

Fig. 3.- UEA-I-HRP. This showed located more intense staining in the perinuclear and basal cytoplasm. x250.

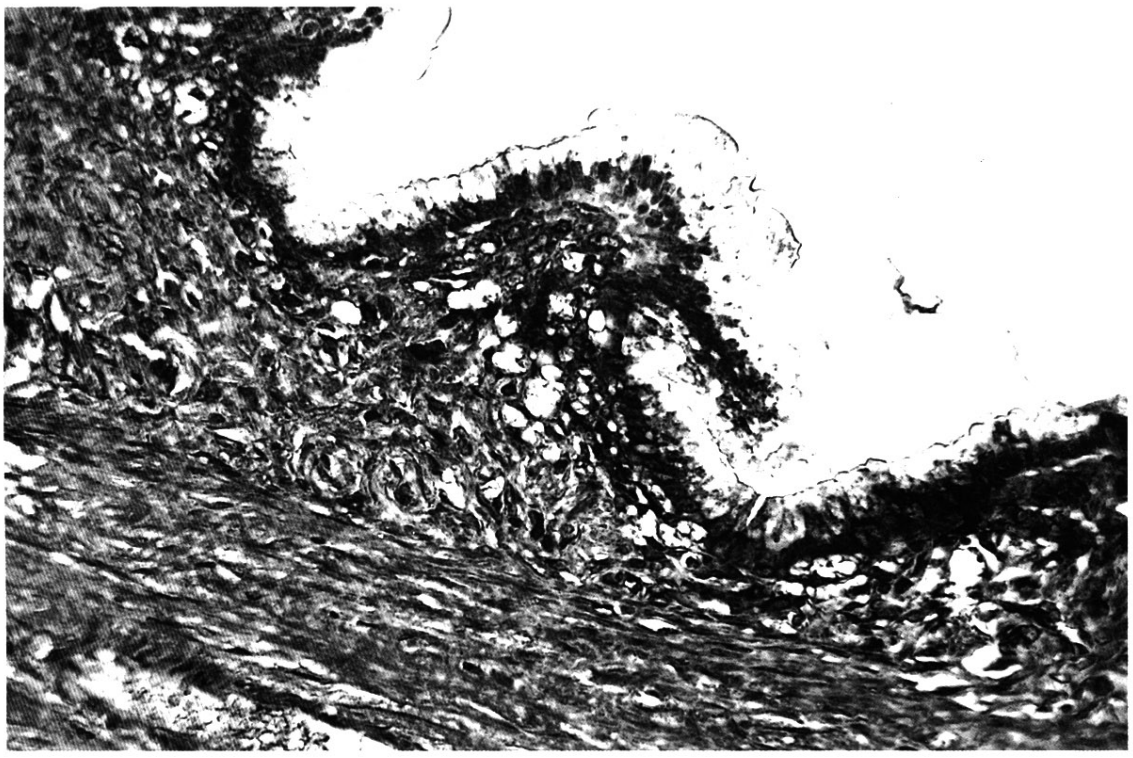

Fig. 4.- ECA-HRP. Positive staining is seen both in the epithelium and the glandular tissue. x250.

dular crypts were positive for this lectin, affording a positive-negative, gland-epithelium pattern (Fig. 1). Con-A binding was more intense at glandular fundus level and was marked in the basal and supranuclear cytoplasm. The glandular neck and goblet cells were negative for this lectin.

With WGA lectin, we observed an alternative pattern of positive and negative staining at superficial and glandular epithelium level, the sugar residues of N-acetylglycosamine being located in the intracellular cytoplasm. WGA binding was located in the supranuclear and superficial edge of the enteric epithelium. The fundus and neck of the glandular crypts had alternative zones of reactivity, while goblet cells were negative.

Table 2.- Intestinal type prismatic epithelium.

	Con-A	WGA	DBA	UEA	VVA	ECA	PHA-P	PNA
superficial epithelium	+/-	+/-	-	-	+++	++	+/-	-
glandular fundus	+++	+/-	-	+++	-	++	+/-	+++
glandular neck	+	+/-	-	+++	-	++	+/-	-
goblet cells	-	-	++	++	-	-	+/-	-

(-) negative staining; (+/-) very weak staining; (+) weak staining; (++) intense staining; (+++) very intense staining.

Table 3.- Localisation of reactivity in epithelial cells.

	ConA	WGA	SBA	VVA	DBA	ECA	UEA	PNA	PHA
Prismatic epithelium	+/-*	+/-		+	+/-*	+*	-*		+/-
Pericellular	+	+		+	+	+/-	+		+
Intracellular		+		+					
Perinuclear	+					+		+	
Basal cytoplasm				+		+			+
Apical cytoplasm				+/-					+
Mucosa epithelium	+*			-/+*	-*	+*	+*		+/-
Pericellular				+				+	
Intracellular	+					+	+	+	
Perinuclear	+					+	+		
Basal cytoplasm	+					+	+		
Apical cytoplasm									

(-) negative staining; (+/-) very weak staining; (+) weak staining; (++) intense staining; (+++) very intense staining; * isolated positive-negative cells.

DBA binding sites marked a line, which was located at the apical pole of the enteric epithelium, staining the apical membrane. Some samples showed reactivity in isolated cells of the epithelial cytoplasm and in the glandular crypts, as well as in the goblet cells of the epithelium (Fig. 2). Thus, the residues of N-acetylgalactosamine were observed very discretely in the enteric epithelium.

The lectin binding for L-fucose, with UEA-I-HRP, was negative in the superficial enteric epithelium. However it marked the free edge of the cells. Positive binding was observed in the glandular crypts from their beginning until their final conformation. The glycidic residues appeared located strongly in the perinuclear and basal cytoplasm (Fig. 3).

Lectin VVA stained the superficial enteric epithelium both intracytoplasmically and in the apical edge. The glandular crypts were negative for this lectin, although there were zones of isolated reactivity at the glandular neck marking the cellular membranes.

The PNA lectin reactivity showed a selective binding of the sugar residues of N- acetylgalactosamine/galactose at the level of the glandular crypt fundus. In the perinuclear cytoplasm of some superficial cells there was a weak binding in isolated cells. Incubation with lectin PHA-P reported alternating positive-negative zones in the epithelium surface, as well as in the glandular epithelium, in the basal cytoplasm, the apical cytoplasm or in the free edge of the epithelium.

By using lectin ECA, positive results were observed both in the epithelium and glandular tissue, being more marked in the basal and perinuclear cytoplasm (Fig. 4). Goblet cells did not appear stained for this lectin.

The vermiform appendix found within the organoid structures of the teratomas showed a similar reaction for the lectins to that described for the enteric epithelium.

Discussion

There are few works on teratomas and none of them makes reference to epithelial tissue. Our results show that this type of tumour can be used as a useful tool to study the changes in differentiation at biochemical level.

In the samples of intestine with prismatic epithelial tissue, labelling with UEA-I revealed a substantial presence of L-fucose receptors in the proximal colon, and the absence of staining in the distal and final portions of the colon, in agreement with the findings of other authors (Shanti and Notter, 1989; Lehtonen et al., 1989). Studies on the primate colonic mucosa have disclosed increases in the glycidic changes in healt-

hy mucosa as compared with inflamed or tumoural mucosa. Fucose radicals tend to increase in chronic inflammatory intestinal disease and were very pronounced in carcinoma (Miyauchi et al., 1882; Hakomori, 1985; Moore et al., 1988). However, fucose radicals may mask the MUC-1 epitope both in normal and in carcinomatous gastric mucosa because the glycidic chain is usually located on the arginine residues of this epitope. The presence of fucose in the fundus and glandular neck was much more striking, as was also the case of goblet cells. We believe that this could be related to both an early and anomalous development of the teratomous tissue and to changes and functional disorders in the coupling of the products of muscle tissue, in agreement with the findings of Andrews (1988).

Labelling with DBA, which is specific for the residues of sugar of the NacGal-1-3 NacGal and NacGal type, was only positive at the level of the goblet cells. The binding was present in intestinal goblet cells, as reported previously (Bresalier et al., 1985; Andrews, 1987; Vance et al., 1988; Jass et al., 1994; Brinck et al., 1995). The decrease in reactivity to the lectin DBA in these goblet cells has been related to several pathological conditions, including hyperplasic polyps and the dysplasias related to adenocarcinomas or carcinomas. Under normal conditions, this lectin shows a very similar pattern in the vermiform appendix and in the proximal portion of the large bowel and changes its labelling at distal and caecum level (Bresalier et al., 1985). Binding for DBA increases in goblet cells of the epithelium and in the glandular crypts of the large bowel, with a decrease in reactivity towards the inside of the crypts and showing great specificity for highly differentiated cells (Jass et al., 1994; Brinck et al., 1995).

PNA was mainly found at the bottom of the crypts, in the basal and perinuclear positions. This finding is consistent with those of other authors who reported the location of the PNA-specific Gal-NacGal dimer at supranuclear level of the glandular crypts (Cooper, 1982; Vance et al., 1988). Some authors have reported that galactose residues, which are specific in their binding to PNA, tend to decrease in normal mucosa when neuraminidase is present (Andrews, 1987). Several papers have described differences in PNA labelling during the evolution of neoplastic disease, showing a fine intracytoplasmic granulation in the dysplastic elements. Accordingly, these authors considered the hypothesis that this group of glycoproteins has possible antigenic potential with interactions between the tumour and the host. Thus, labelling with PNA could serve as a signal of neoplastic differentiation and tumour progression (Cooper, 1982; Miyauchi et al., 1982; Hakomori, 1985; Fenderson et al., 1987; Moore et al., 1988). Additionally, labelling with PNA, located at supranuclear level under normal conditions, tends to reduce this localisation and to appear apically in oncological processes (Cooper, 1982). Similar results have been found in the early diagnosis of dysplastic differentiation in human colonic adenocarcinoma. The increase in PNA labelling in chronic intestinal disease of the colon and in premalignant changes has been interpreted in terms of dietary influences (Moore et al., 1988). Other authors have proposed that PNA could behave as a mitogen in the human colonic epithelium via binding to diverse receptors. Thus, different lectins, such as PNA, could promote proliferation and neoplastic growth. By contrast, the glalactose ingested in the vegetables of the diet could inhibit this effect via a competitive effect with the PNA (Schachner, 1982). In gastric inflammatory processes the contribution of the bacterium *Helicobacter pylori* has been related to an increase in reactivity to PNA (Rameshkumar et al., 1994).

Residues of Nac-glucosamine, galactose and Nac-galactosamine were observed on the surface of the neck and the glandular fundus. These were quantitatively variable and were located in the cell membrane and at intracytoplasmic level. These residues displayed a complex localisation, distribution and intensity. The differences could likely reflect immaturity and disturbances in cell junctions during development, as proposed by King in 1985.

In some cells of the epithelium and neighbouring glandular tissue, labelling with PHA-P revealed a low intensity cellular pattern. However, the receptors for PHA-P increase during processes of malignant transformation of the epithelium, above all in gastric carcinoma (Miettinen et al., 1985).

Concerning dietary environmental influences, reports have been made of changes in goblet cells, both due to the ingestion of certain diets and due to the intervention of microflora. Decreases in sialic acids bound to glalactose and fucose have been reported for conventional diets with respect to purified ones (Sharma and Schumacher, 1995). The consumption of food rich in polyunsaturated fatty acids seems to decrease labelling to the lectin WGA in the microvilli of the ileum and jejunum (Alessandri et al., 1995). Other studies have related the progressive changes that occur in celiac diseases to the binding of mannose-glucose sugars, Nacglc and NacGal to the intracytoplasmic protein fraction of gliadin (Pittschieler et al., 1994).

In stomach carcinoma, the binding sites of NacGal to the lectins SBA and DBA increase (Chen, 1989). This glycidic residue, also detected with the lectin MLL, is used to differentiate non-proliferative cell populations in the intestinal tract of the mouse. Normally, MLL labels the cells that differentiate into goblet cells and are seen to migrate towards the crypt (Falk et al., 1995).

The terminal trisaccharide Sialic2-6Gal1-4NacGal has been detected histochemically by use of

the lectin TFA in the differentiation of malignant adenocarcinoma (Yamashita et al., 1995). Many authors have detected change in sialic components in certain pathological processes in which losses of the oxy-acetylation of sialic acids in carbons 4,7,8 and 9, together with an increase in sialization are seen (Ahnen et al., 1987). However, these changes have not been detected in colon adenocarcinoma (Jass and Roberton, 1994). Accordingly, not all the changes observe din the glycidic components seem to be related to malignant processes.

In conclusion, our findings are in close agreement with those of other studies, both as regards normal conditions and pathological states for the residues of fucose, mannose-glucose, Nacglc and NacGal. However, teratomas develop under conditions in which the glycoproteins components of epithelial cells are not related to environmental influences during differentiation, maturation and transformation. By contrast, the glycidic changes must be related exclusively to the actual genotypic potential of the tissue. We believe that many of the glycidic changes observed in the intestinal processes could be related to the phenotypic expression of the tissue instead of reflecting different extrinsic factors.

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