Glycoconjugate Journal 20, 367–374, 2004

Lectin histochemistry of normal human gastric mucosa

Chong Jiang, Sheena F. McClure, Robert W. Stoddart and John McClure

Laboratory Medicine Academic Group, School of Medicine, University of Manchester, Oxford Road, Manchester M13 9PT

Information about the saccharides expressed in gastric mucosa is mostly limited to the glycan content of gastric mucins and there are only a few studies of the glycoprofiling of the constituent cells and their components. Knowledge of the glycan expression of normal gastric mucosa is necessary for the interpretation of the significance of changes of expression in disease.

A lectin histochemical study of normal human gastric (body) mucosa was performed using 27 lectins chosen to probe for a wide range of oligosaccharide sequences within several categories of glycoprotein glycans.

There were marked differences in staining reactions in the various microanatomical structures of the mucosa, particularly between pits and glands with the former more closely resembling the surface epithelium. A notable feature was the degree of difference in the staining between a substantial sub-population of cells within the neck region and the epithelium of both the pits and glands. These neck cells resembled the pit cells with some lectins, glandular cells with some others and neither with some other lectins. Overall, the differences between the pit, gland and neck epithelia were diverse and numerous, and could not be explained by altered activity of a small set of glycosyltransferases. Widespread alterations of glycans must have occurred (affecting terminal and internal parts of their structures) and the very different glycotypes of the pit, neck and gland epithelia are, therefore, suggestive of the existence of three cell lineages within normal gastric epithelium.

Published in 2004.

Keywords: **gastric mucosa, lectin, glycan**

Introduction

Glycans are widely regarded as being indicators of cellular differentiation and some are known differentiation antigens [1]. Structural information about the saccharides expressed in normal human gastric mucosa, in relation to the histology of the organ and its cell lineages, can be gained by the use of lectin histochemistry, but previous studies have been limited because of their use of a small number of lectins. Available information relates mostly to the glycan content of gastric mucins and there are few studies on the glycotyping of the cells and their constituents [2–5]. In order to obtain information about normal tissues, which is necessary for the analysis and interpretation of glycan expression in gastric diseases, a study was made of the binding of twenty-seven lectins to normal human gastric mucosa. These lectins were selected to probe for a wide range of oligosaccharide sequences within several categories of glycoprotein glycans and they represent the most comprehensive set of such probes yet to be applied to normal gastric mucosal tissue (Table 1).

To whom correspondence should be addressed: Professor J. McClure, MD, FRC Path, Directorate of Laboratory Medicine, Central Manchester and Manchester Children's University Hospitals NHS Trust, Oxford Road, Manchester M13 9WL, UK. Tel: +44 161 276 8945; Fax: +44 161 276 8089; E-mail: john.mcclure@man.ac.uk

Materials and methods

Fifteen formalin-fixed, paraffin-embedded specimens of grossly normal human gastric (body) mucosa were drawn from the diagnostic tissue archive. Sections (4 μm) were cut onto APES (3-aminopropyltriethoxysilane)-coated slides, dewaxed in xylene and taken to ethanol. Endogenous peroxidase was blocked in methanolic hydrogen peroxide and the sections were then hydrated through graded ethanols to water. They were pretreated with a solution of 0.1% (w/v) trypsin (type II crude, from porcine pancreas, Sigma Chemical Co.) in 0.05 M TRIS buffered saline, pH 7.6 containing 0.1% (w/v) calcium chloride. They were then stained with the panel of 27 biotinylated lectins, using a lectin-biotin avidin-peroxidase method established and used in our laboratory [6], with DAB as the disclosing agent and a methyl green counterstain. Most of the biotinylated lectins were obtained from the Sigma Chemical Company, MAA and

Table 1. The origins and sugar binding properties of the lectins used in this study

Lectin group	*Acronym*	*Origin*	*Sugar specificity*
1	GNA	*Galanthus nivalis*	Non-reducing terminal α-*D*-mannose, preferentially mannosyl α1,3 mannose linkage
	NPA	*Narcissus pseudonarcissus*	Non-reducing terminal & internal α-*D*-mannose, preferentially mannosyl α1,6 mannose linkage
	PSA	*Pisum sativum*	α-*D*-Mannose in non-bisected bi/tri-antennary complex *N*-linked sequences especially with core fucosylation
	LCA	*Lens culinaris*	α-*D*-Mannose residues, similar but not identical to PSA
	e-PHA	*Phaseolus vulgaris*	Bi/tri-antennary, bisected complex *N*-linked sequences
	l-PHA*	*Phaseolus vulgaris*	Tri/tetra-antennary, non-bisected complex *N*-linked sequences
2	UEA-I*#	*Ulex europaeus*	α-*L*-Fucosyl terminals, especially LFucα1,2Galβ1,4GlcNAcβ1-
	LTA#	*Tetragonolobus purpureus*	α-*L*-Fucosyl terminals, especially where clustered on core sequences of *N*-glycans
3	SNA*#	*Sambucus nigra*	NeuNAcα2,6Gal/GalNAcβ1-
	MAA*#	*Maackia amurensis*	NeuNAcα2,3Galβ1-
	LFA#	*Limax flavus*	NeuNAcα2,3/6Galβ1- > NeuNGl-
4	PNA/AHA*#	*Arachis hypogaea*	Galβ1,3GalNAcα1- > Galβ1,4GlcNAcβ1-
	ECA*#	*Erythrina cristagalli*	Galβ1,4GlcNAcβ1-
	CTA	*Erythrina corallodendron*	Galβ1,4GlcNAc-, especially in multiple branches
5	HPA*#	*Helix pomatia*	GalNAcα1-
	WFA*#	*Wisteria floribunda*	GalNAcα1,6Galβ1- > GalNAcα1,3Galβ1-
	VVA*#	*Vicia villosa*	GalNAcα1,3Galβ1- > GalNAcα1,6Galβ1-
	SBA*#	*Glycine max*	GalNAcα1,3- > Galα1-
	PTA#	*Psophocarpus tetragonolobus*	GalNAcα1- > Galα1-
	MPA#	*Maclura pomifera*	Galβ1,3GalNAcα1- > GalNAcα1-
	BPA*#	*Bauhinia purpurea*	Galβ1,3GalNAcα1-
	DBA#	*Dolichos biflorus*	GalNAcα1,3(LFucα1,2)Galβ1,3/4GlcNAcβ1-
6	STA	*Solanum tuberosum*	(-4GlcNAcβ1-)$_n$- > (-3Galβ1,4GlcNAcβ1-)$_n$
	LEA	*Lycopersicon esculentum*	(-4GlcNAcβ1-)$_{2-4}$
	DSA*#	*Datura stramonium*	Galβ1,4GlcNAcβ1- > (-4GlcNAcβ1-)$_2$
7	BSA-IB$_4$#	*Griffonia simplicifolia*	Galα1,3Galβ1,4GlcNAcβ1-
8	BSA-II#	*Griffonia simplicifolia*	GlcNAcα1-

*Means neuraminidase digestion and # means β-elimination were also carried out.

SNA were from Boehringer Mannheim and GNA, NPA, LFA and STA were from EY Laboratories (San Mateo, USA). All were applied at a concentration of 10 μg/ml apart from MAA and SNA, which were used at 50 μg/ml. Negative control sections were included in every staining run with buffer replacing the lectin and, where possible, controls were carried out using the appropriate competing sugars. Positive controls were also carried out, variously by using other tissues within the section or by using known 'positive' slides from blocks of other tissues. Digestion with neuraminidase (neuraminidase VI from *Clostridium perfringens*, Sigma Chemical Co.) was performed, on additional sections, at 0.1 U/ml in 0.2 M sodium acetate buffer (pH 5.5) containing 1% (w/v) calcium chloride at 37°C for 1 h, prior to staining with 12 of the 27 lectins. This digestion was used as a control for the staining by SNA and MAA and also to determine the sub-terminal sugars. Beta-elimination was performed, before staining with 18 of the lectins, based on the method of Downs *et al.* [7], as follows: the sections were incubated in 0.17 M potassium hydroxide containing 50% (v/v) dimethylsulphoxide and 10% (v/v) ethanol at 45°C for 1 h, neutralised in 10 mM hydrochloric acid and then washed in 0.1 M sodium phosphate buffer (pH 7).

Staining intensity was ranked using (−) for negative, (+) for just detectable, positive staining, (++) for moderately positive clear staining and (+++) for strongly positive staining.

The blood groups of the cases were determined from the staining patterns of the erythrocytes of blood vessels initially identified within the tissues by their refractility. The optical system was then adjusted to eliminate refraction artefact and true lectin staining was determined. The blood groups were indicated by staining with the lectins HPA (group A), DBA (group A_2), BSA-IB$_4$ (group B) and UEA-I (group O) [8].

Results

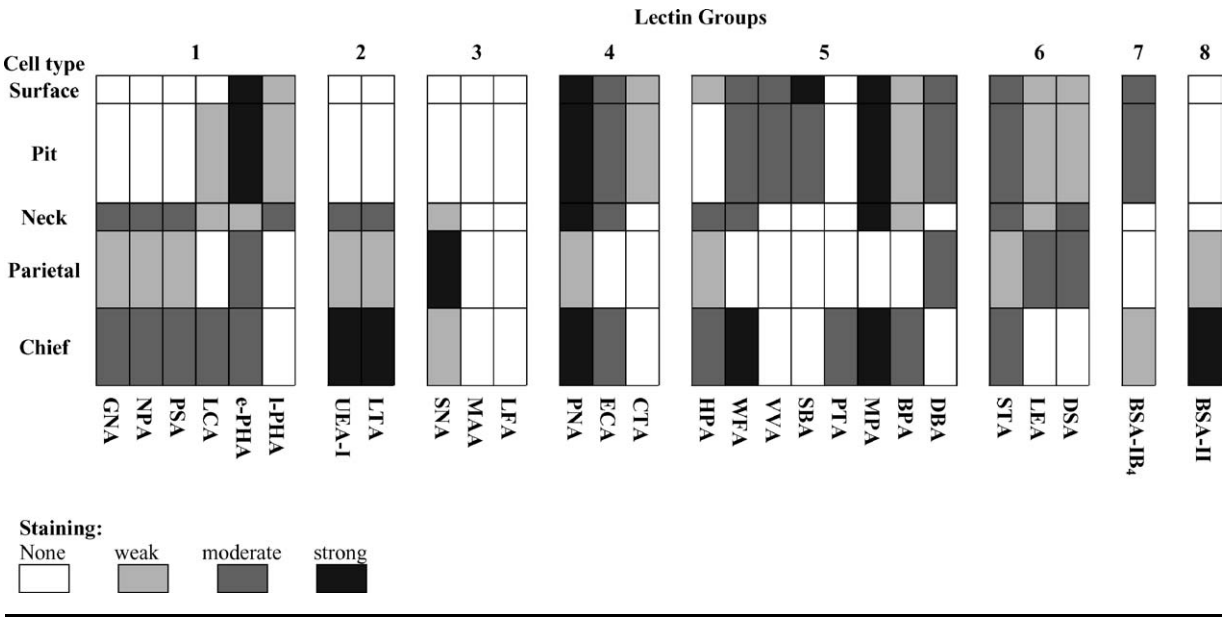
The results are detailed in Table 2 and summarised diagrammatically in Table 3. To facilitate understanding of the results, Figure 1 is a diagrammatic representation of the microanatomy of the mucosa of the body of the stomach. The surface refers to the top layer of mucosal epithelium, the pit epithelium is

Table 2. Summary of the results of the lectin staining of normal human gastric tissue

				Gland cells			
Lectins	*Surface epithelial cells*	*Pit cells*	*Neck cells*	*Parietal cells*	*Chief cells*	*Stroma*	*Blood vessels*
GNA	−	−	++	+	++	−	−
NPA	−	−	++	+	++	−	−
PSA	−	−	++	+	++	+++	+++
LCA	−	+	+	−	++	++	−
e-PHA	+++	+++	+	++	++	+++	+++
l-PHA	+	+	++	−	−	+	−
UEA-I	−	−	++	+	+++	−	−
LTA	−	−	++	+	+++	−	−
SNA	−	−	+	+++	+	++	−
MAA	−	−	−	−	−	−	−
LFA	−	−	−	−	−	++	++
PNA	+++	+++	+++	+	+++	−	−
ECA	++	++	++	−	++	++	++
CTA	+	+	−	−	−	−	−
HPA	+	−	++	+	++	−	+
WFA	++	++	++	−	+++	−	−
VVA	++	++	−	−	−	−	−
SBA	+++	++	−	−	−	−	−
PTA	−	−	−	−	++	−	−
MPA	+++	+++	+++	−	+++	+++	+++
BPA	+	+	+	−	++	++	++
DBA	++	++	−	++	−	−	−
STA	++	++	++	+	++	++	++
LEA	+	+	+	++	−	+	+
DSA	+	+	++	++	−	++	++
BSA-IB_4	+++	+++	−	−	+	−	−
BSA-II	−	−	−	+	+++	−	−

Intensity of staining: − = none, + = weak, ++ = moderate, +++ = strong.

Table 3. The staining patterns of normal human gastric mucosa with various lectins



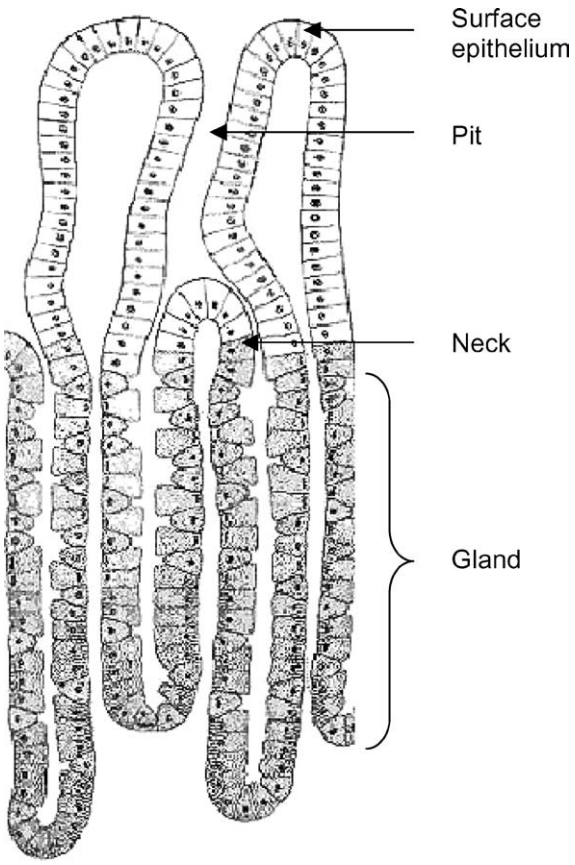


Figure 1. Diagrammatic representation of the mucosa of the body of the stomach.

the part between the surface and the gland, the neck cells are those near the opening of the glands into the pits and the gland epithelium refers to the part from the neck zone to the bottom of the glandular structures.

Marked differences in staining reactions were found between the epithelia of the pits and of the glands, as was expected (see Figure 2a and b). The surface epithelium closely resembled that of the pits, with some small variations i.e. LCA weakly stained the pit cells, but not the surface cells, HPA stained the surface cells and only a few pit cells and staining by SBA was stronger on the surface (Table 2). However, a notable feature was the degree of difference in the staining between a substantial subpopulation of cells within the neck region and the epithelia of both the pits and the glands. These neck cells resembled the pit cells in some cases, glandular cells in others and neither in some.

Staining of the chief cells was more intense than that of the parietal cells with 16 of the lectins, the differences being largely in cytoplasmic staining (see Figure 3 with MPA): the converse pattern was seen less often with four lectins being selective for parietal cells in the glands. Occasional 'positive' cells were seen among generally 'negative' populations in the surface epithelium (with PSA, LCA, MAA, and PTA), in the pits (with PSA, UEA-I, MAA, HPA and PTA), in the neck cells (with MAA, SBA, PTA and DBA) and in the glands (with l-PHA, MAA, LFA, VVA and SBA); it was difficult to identify the precise types of these cells. Lectin binding generally increased in the pit and gland epithelia up to a short distance from the neck cell region and remained constant at greater distances. The position at which this maximal staining started varied a little between lectins.

The results of neuraminidase pre-digestion and β-elimination are shown in Tables 4 and 5, respectively. There was decreased staining with SNA and MAA after neuraminidase treatment, confirming the specificity of the lectin binding. DSA was the only lectin that demonstrated a marked increase in staining intensity after neuraminidase treatment. The changes after neuraminidase in lectin binding of the surface and pit

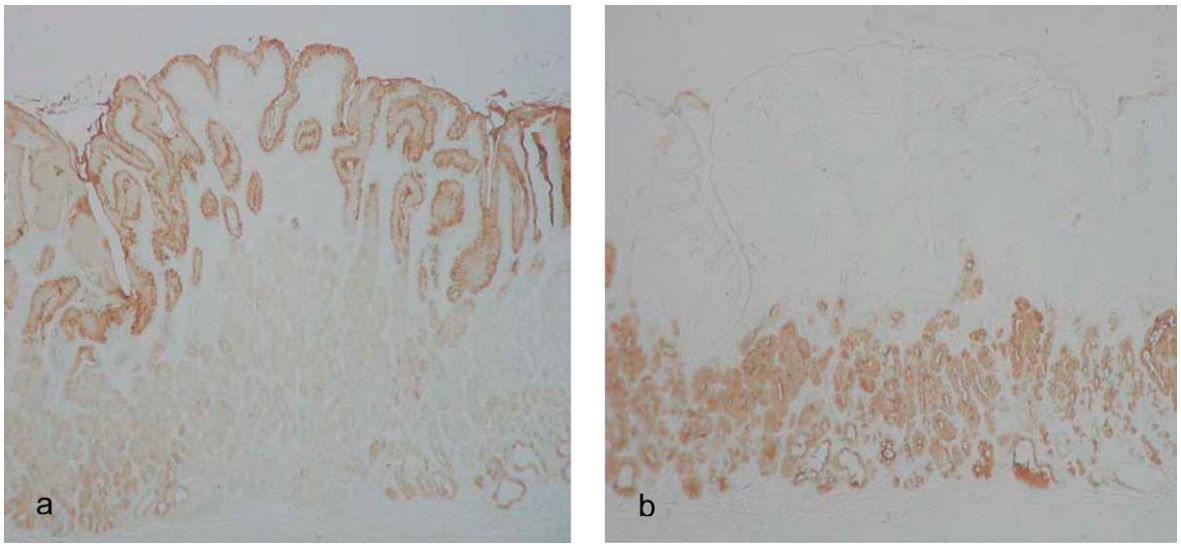


Figure 2. Lectin staining of normal gastric mucosa. (a) The lectin BSA-IB_4 stained the surface and pit epithelium but only a few cells of the glands. (b) BSA-II stained the glands, but not the surface and pit epithelium.

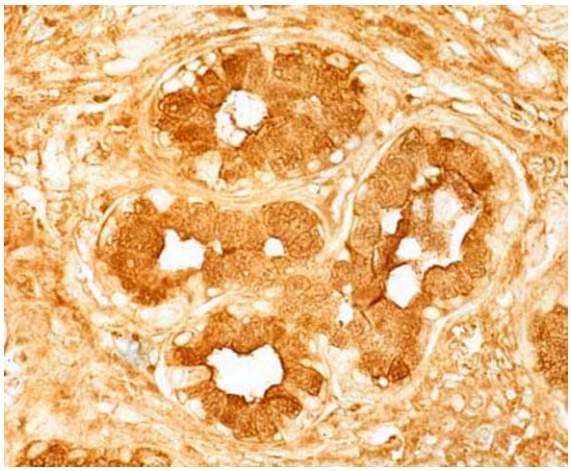


Figure 3. Lectin staining of gland cells of normal gastric mucosa MPA stained chief cells but not parietal cells.

Table 4. Changes in the level of lectin staining of gastric mucosa after neuraminidase digestion

Lectin	*Surface epithelial cells*	*Pit cells*	*Gland cells*
l-PHA	=	=	=
UEA-I	−	=	=
SNA	−	−	↓
MAA	↓	↓	↓
PNA	↓↓	↓↓	↓
ECA	↓	↓	=
HPA	↑	↑	=
WFA	↓	↓↓	↓
VVA	↓	↓	=
SBA	↓↓	↓↓	=
BPA	=	=	=
DSA	↑↑	↑↑	↑↑

'↑' refers to an increase and '↓' to a decrease of staining. '=' refers to no change and '−' to no staining either before or after digestion.

cells were similar (changes for these with SBA are shown in Figure 4a and b) and the changes in the neck cells were similar to those of the gland cells. Staining levels were slightly diminished with eight of the lectins after β-elimination, with more marked loss of staining with LTA in gland cells and with SBA in surface epithelial cells.

The blood group status of the different cases had no influence on the staining pattern of the epithelia.

Discussion

From all the observations reported here it can be concluded that the 'high mannose' N-glycans were largely restricted to the neck cells and the chief cells of the glands (GNA and NPA). The complex N-glycans occurred much more generally, with the 'bisected' subset being most abundant in the surface and pit epithelia (e-PHA), the 'non-bisected' subset being mostly in the neck cells and chief cells of the glands (PSA and LCA) and the tetra-antennary forms being mostly in the neck cells (l-PHA). Terminal α-L-fucosyl residues (detected by UEA-I and LTA) were found in the gland and neck cells, but not on the surface or pit epithelia, except where they were co-expressed with terminal 2-deoxy,2-acetamido-α-D-galactose (*N*-acetylgalactosamine), as detected by DBA, when the surface and pit epithelia also showed expression. Terminal *N*-acetylneuraminic acid expression, whether in the α2,6 or α2,3 linkage, was mostly restricted to neck cells with some scattered positive cells in other areas (SNA and MAA).

A complex pattern of glycans with β-galactosyl termini was shown by the staining with PNA, ECA, CTA, STA, LEA and

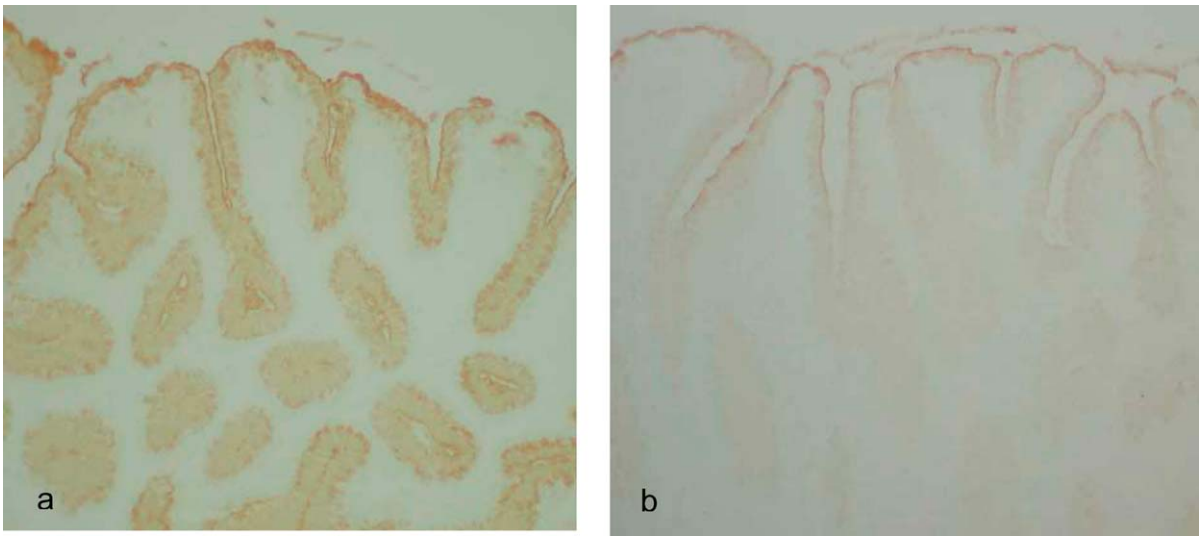


Figure 4. Effect of neuraminidase pre-treatment on staining of gastric mucosa (a) and (b). Staining with SBA (a) before and (b) after neuraminidase pre-treatment.

Table 5. Changes in the level of lectin staining of gastric mucosa after β-elimination

Lectin	*Surface epithelial cells*	*Pit cells*	*Gland cells*
UEA-I	–	=	↓
LTA	↑	–	↓↓
SNA	–	–	=
MAA	↑	=	=
LFA	–	–	=
PNA	=	=	=
ECA	=	=	↓
HPA	=	=	=
WFA	=	↓	↓
VVA	=	=	↓
SBA	↓↓	↑	=
PTA	↓	↓	↓
MPA	=	=	=
BPA	↓	↓	↓
DBA	↓	↓	=
DSA	=	=	=
BSA-IB_4	=	=	↓
BSA-II	–	–	=

'↑' refers to an increase and '↓' to a decrease of staining. '=' refers to no change and '–' to no staining either before or after treatment.

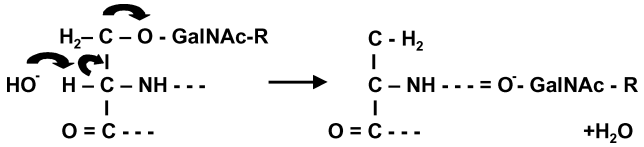


Figure 5. Beta elimination of the *O*-glycosyl serine linkage. The *O*-glycosyl threonine linkage degrades in a similar manner.

DSA. Much of the variability (e.g. between ECA and CTA) probably reflects different branching patterns in the inner sequences of these glycans. Terminal β-galactose is, however, a widespread feature of glycans of all the cell types examined. A complex pattern in the distribution of glycans terminating in 2-deoxy,2-acetamido-α-D-galactose was shown by the lectins of group 5; the α1,3 linkage appeared to be restricted mostly to the pits (SBA and VVA), with the α1,6 linkage being more general (WFA). A comparison of the staining by PNA, MPA and BPA is indicative of heterogeneity in glycans carrying terminal sequences of Galβ1,3GalNAc-.

Alpha-galactosyl termini were found mostly in the mucous epithelium of the surface and the pits (BSA-IB_4) and terminal 2-deoxy,2-acetamido-α-D-glucose (*N*-acetylglucosamine) was found only in the glands (BSA-II).

In addition to the direct observations of lectin staining, pre-digestion with neuraminidase or pre-treatment using β-elimination provided further insights. Neuraminidase hydrolyses terminal sialyl residues from glycans, so exposing sub-terminal sequences for detection and analysis by lectin binding. Therefore, if terminal sialyl residues were present, any differences in staining pattern after digestion would provide more structural information. The increase in staining of all cell types with DSA after neuraminidase suggests that the glycan which binds to DSA was blocked by a sialyl residue, and, perhaps, was present on similar oligosaccharides occurring in all cell types. The surface and pit epithelia responded in a similar manner to neuraminidase, suggesting similarities in the cell types. With β-elimination, alkali-labile glycans, O-glycosidically linked to serine and threonine via α-*N*-acetylgalactosamine, are cleaved; the O-glycan is removed leaving an unsaturated bond in the amino acid (Figure 5). By comparing the lectin staining patterns of sections of the same tissue, variously exposed or not exposed to conditions for β-elimination, the content of O-glycans in tissues and their contribution to lectin binding can be explored [7]. In this study there was some decrease in staining after β-elimination suggesting that there were some O-glycans present, but that the majority of those binding to lectins were N-glycans.

Previous studies of normal human gastric mucosa by Ito *et al.* [2] found that the lectin DBA stained specific parts of the surface epithelial and parietal cells and Kessimian *et al.* [3], looking particularly at parietal cells, showed strong staining with BSA-IB_4, DBA, PNA and SBA. Malchiodi-Albedi *et al.* [9] studied fundic glands using only PNA and showed staining of parietal cells, chief cells and mucous neck cells suggesting that this lectin could be used as a marker for the secretory canaliculi of parietal cells. Narita and Numao [5] studied gastric surface mucosa with five lectins, ConA, WGA, PNA, UEA-I and DBA, all of which were positive and which agree with our results apart from those given by UEA-I. The lack of binding of UEA-I to the surface mucosal epithelium, in the present study, despite its binding to some of the pit epithelium (which acts as an internal control) and the positive staining of the surface mucosal epithelium with DBA, suggests that all type II chains with fucosyl residues attached by α1,2 linkages, in the surface mucosae of the tissues studied here, were also substituted by α1,3-linked 2-deoxy,2-acetamido-galactosyl residues. This does not preclude the possibility that fucosylated type I chains might be present, expressing the H-antigen or related antigens, as in the studies of Madrid *et al.* [10], since no probe specific for H-antigens in type I chains was applied here and UEA-I is specific for type II chains. The lack of stain with LTA suggests that clustered fucosyl residues are generally absent, except, possibly, in the neck cells. In the present study, there was no association with expression of A, B and H-antigens and no case was shown to be a blood group secretor.

The patterns of staining with the lectins used here are consistent with there being two cell lineages which originate from stem cells located in the neck region, which migrate away from each other towards the surface and the glands, respectively [11–13]. Their mature glycotypes appear to be fully expressed within a short distance (signifying only a few cells) of the neck region, though with apparent slight differences in this distance between

the lectin ligands. Careful study with accurately aligned tissues is required to confirm this.

A third population of cells in the neck region has previously been identified [2,12,13] and appears to correspond to the distinctive population of neck cells seen here. These cells have been considered as transit cells lying between the gastric stem cells and the chief cells that contain pepsinogen [11,12], but they have also been shown to express trefoil peptides [14,15] and it has, therefore, been suggested that they are a separate, mature, cell lineage with a specific function in mucosal protection and repair [12]. If this is so, the mucous neck cells of the stomach would be predicted to have a glycotype similar to the cells of Brunner's glands of the duodenum. There is a morphological similarity between these cells [11], but the secretions are different; Brunner's glands produce a clear alkaline mucus (pH 8.2–9.3) with a high content of bicarbonate and neck cells produce a less alkaline mucus [12]. Hughes *et al.* [13] showed that both cell types stain strongly and preferentially with a monoclonal antibody to antigen D10. Both cell types also produce trefoil peptides [15]. The pattern of lectin histochemical staining of Brunner's glands was very similar to that of the ulcer-associated cell lineage [16] and both were similar, including subcellular distribution, to that found in the neck cells in this study, with a few variations. These variations were, first: stronger staining of neck cells than the cells of Brunner's glands with GNA, second: e-PHA and LEA stained neck cells, but not Brunner's glands, and third: there was staining by the lectins CTA, VVA and BSA-IB_4 of Brunner's glands, but not neck cells. These represent only six points of difference out of twenty-three comparisons. The difference with e-PHA could represent a variation in the activity of 2-deoxy,2-acetamido-glucosyl transferase III, which determines bisection in complex *N*-glycans [17]. The differences with CTA, VVA and BSA-IB_4 could arise from variation in three transferases, or fewer if internal sequences near the non-reducing termini are altered. The difference with GNA was purely of degree and may well reflect a difference in the number of secretory vesicles.

The expression of certain glycans (the so-called differentiation antigens) changes as cells mature. This can lead to a gradation of staining intensity from that found in one cell population towards that found in another cell population. The chief and parietal cells of the glands are fully differentiated cells and should have stable glycotypes, but their less mature precursors will show a range of staining reactions. Overall, in this study, the differences in the staining patterns between the surface and pit epithelia were small and could be explained by minor changes in the transferases which add 2-deoxy,2-acetamido-α-D-galactose and the bisecting 2-deoxy,2-acetamido-α-D-glucosyl residue. The differences between the pits and the glands, and between each of these and the neck cells, were much more diverse and numerous, and could not be explained by the altered activity of a small set of glycosyltransferases. Widespread alteration of glycans must have occurred, affecting both terminal and internal parts of their structures. The very different glycotypes of the pit, neck and gland epithelia are, therefore, powerfully suggestive of the existence of three cell lineages within gastric epithelium.

Acknowledgments

We gratefully acknowledge the secretarial support of Mrs PJ Bellinger.

References

1 Sharon N, Lis L, Glycoproteins: Structure and function. In *Glycosciences: Status and Perspectives*, edited by Gabius H-J, Gabius S (Chapman and Hall, Weinheim, 1997), pp. 133–62.
2 Ito M, Takata K, Saito S, Aoyagi T, Hirano H, Lectin-binding pattern in normal human gastric mucosa: A light and electron microscopic study, *Histochemistry* **83**, 189–93 (1985).
3 Kessimian N, Langner BJ, McMillan PN, Lectin binding to parietal cells of human gastric mucosa, *J Histochem Cytochem* **34**, 237–43 (1986).
4 Kuhlmann WD, Peschke P, Wurster K, Lectin-peroxide conjugates in histopathology of gastrointestinal mucosa, *Virchows Arch A Pathol Anat Histopathol* **398**, 319–28 (1983).
5 Narita T, Numao H, Lectin binding patterns in normal, metaplastic, and neoplastic gastric mucosa, *J Histochem Cytochem* **40**, 681–7 (1992).
6 Jones CJP, Stoddart RW, A post-embedding avidin-biotin peroxidase system to demonstrate the light and electron microscopic localisation of lectin binding sites in rat kidney tubules, *Histochem J* **18**, 371–9 (1986).
7 Downs F, Herp A, Moschera J, Pigman W, Beta-elimination and reduction reactions to some applications of dimethylsulfoxide on submaxillary glycoproteins, *Biochim Biophys Acta* **328**, 182–92 (1973).
8 Boyd WC, Reguera RM, Haemagglutinating substances for human cells in various plants, *J Immunol* **62**, 333–41 (1949).
9 Malchiodi-Albedi F, Barsotti P, Mingazzini P, Marinozzi V, Visualization of the secretory canaliculi of human parietal cells with peroxidase-labelled peanut lectin: Light- and electron-microscopic observations, *Cell Tissue Res* **239**, 447–50 (1985).
10 Madrid JF, Leis O, Diaz-Flores L, Hernandez F, Secretion of fucosylated oligosaccharides related to the H antigen by human gastric cells, *Histochem Cell Biol* **110**, 295–301 (1998).
11 Bloom W, Fawcett, DW, *A Textbook of Histology* (WB Saunders Company, Philadelphia, 1975), p. 652.
12 Fawcett DW, Jensh RP, *Bloom and Fawcett: Concise Histology* (Chapman and Hall, New York, 1997), p. 197 & 206.
13 Hughes NR, Bhathal PS, Francis DMA, Phenotypic identity of gastric mucous neck cells and mucous cells of cardiac, pyloric, and Brunner's glands, *J Clin Pathol* **47**, 53–7 (1994).
14 Alison MR, Chinery R, Poulsom R, Ashwood P, Longcroft JM, Wright NA, Experimental ulceration leads to sequential expression of spasmolytic polypeptide, intestinal trefoil factor, epidermal growth factor and transforming growth factor alpha mRNAs in rat stomach, *J Pathol* **175**, 405–14 (1995).

15 Poulsen SS, Thulsen J, Nexo E, Thim L, Distribution and metabolism of intravenously administered trefoil factor 2/porcine spasmolytic polypeptide in the rat, *Gut* **42**, 240–7 (1998).
16 Roberts ISD, Stoddart RW, Ulcer-associated cell lineage ('pyloric metaplasia') in Crohn's disease: A lectin histochemical study, *J Pathol* **171**, 13–9 (1993).
17 Schachter H, Branching of *N*-glycans: *N*-acetylglucosaminyltransferases. In Carbohydrates in Chemistry and Biology, Part 2: Biology of saccharides, edited by Ernst B, Hart GW, Sinay P (Wiley-VCH, Weinheim, 2000), pp. 145–173.

Received 17 June 2003; revised 26 February 2004;
accepted 1 March 2004