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ORIGINAL ARTICLE

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Mucins as key molecules for the classification of intestinal metaplasia of the stomach

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Abstract Mucins and mucin-associated carbohydrates have a distinct expression pattern that can be modified under pathological conditions. Normal gastric mucosa expresses MUC1 and MUC5AC in foveolar epithelium and MUC6 in the glands. Lewis type-1 chain antigens (Le^a and Le^b) are expressed in foveolar epithelium, whereas Lewis type-2 chain antigens (Le^x and Le^y) are expressed in the glands. In this study we used monoclonal antibodies to evaluate the pattern of mucins and Lewis type-1 carbohydrates in intestinal metaplasia (IM) and compared it with IM types determined using histochemistry. In type-I or complete IM we found expression of MUC2 intestinal mucin and decreased/absent expression of MUC1, MUC5AC and MUC6. In type-II/III or incomplete IM there was co-expression of MUC2 and the mucins expressed in the stomach. No major differences were detected among the three IM types regarding expression of Lewis antigens. Furthermore we observed that sialylated compounds other than sialyl-Le^a are responsible for histochemical detection of sialomucins and that sulpho-$Le^{a/c}$ is expressed in the presence or absence of sulphomucins detected using histochemistry. We conclude that mucin immunohistochemistry may replace classic histochemistry for the classification of IM into complete and incomplete types. The present study challenges the distinction of type-II from type-III IM since we did not observe major differences in the expression profile of mucins and Lewis type-1 carbohydrates. Finally, it seems necessary to evaluate the predictive value of IM according to the presence of specific sulphated carbohydrates (e.g. sulpho-$Le^{a/c}$) rather than histochemically detected sulphomucins.

Keywords Mucins · Lewis type-1 carbohydrates · Intestinal metaplasia · Gastric carcinoma

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Introduction

Mucins are high molecular weight glycoproteins that constitute the major component of the mucus layer that protects the gastric epithelium from chemical and mechanical aggressions. Twelve mucin genes that code for the protein part of mucins (apomucins) have been identified: *MUC1*, *MUC2*, *MUC3*, *MUC4*, *MUC5AC*, *MUC5B*, *MUC6*, *MUC7* [8], *MUC8* [21], *MUC9* [11], *MUC11* and *MUC12* [27]. *MUC2*, *MUC5AC*, *MUC5B* and *MUC6* genes have been mapped on a cluster on chromosome 11p15.5, and *MUC3*, *MUC11* and *MUC12* have been mapped on another cluster on chromosome 7q22 [27]. The presence of a variable number of tandem repeats that code for amino acid sequences rich in serine and threonine residues is a common characteristic of mucins. These amino acid residues are potential *O*-glycosylation sites for attachment of the glycan side chains that constitute ~80% of the molecular weight of the final mucin glycoproteins.

Normal gastric mucosa produces mainly neutral mucins, except for the mucus-secreting cells of the neck glands that secrete acid mucins [5]. Normal human stomach expresses MUC1, MUC5AC and MUC6. MUC1 and MUC5AC are expressed in the superficial foveolar epithelium, whereas MUC6 is expressed in the mucous neck cells of the body and deeper glands of the antrum. Lewis type-1 chain antigens (Le^a and Le^b) are expressed in the surface foveolar epithelium, whereas Lewis type-2 chain antigens (Le^x and Le^y) are expressed in the glands

[15]. De Bolós et al. [4] demonstrated that the zonal expression of mucins and Lewis antigens is tightly associated and showed that MUC5AC-expressing cells also express Lewis type-1 antigen Le^a, whereas MUC6 expressing cells express Lewis type-2 antigen Le^y.

Mucins and mucin-associated carbohydrate chains have a distinct tissue expression pattern that can be quantitatively and qualitatively modified under pathological conditions [1, 2, 9, 10, 13, 18, 19]. Intestinal metaplasia (IM) was characterised at the histochemical level by the abnormal expression of acid mucins (both sialomucins and sulphomucins) [6]. In IM the expression pattern of both mucins and carbohydrates is modified [10, 16, 18]. Mucin expression in IM can be described under two major profiles: one with decreased expression of MUC1, MUC5AC and MUC6 and de novo expression of the intestinal mucin MUC2 (complete IM); and the other with coexpression of MUC1, MUC5AC, MUC6 and MUC2 (incomplete IM) [18].

Carbohydrate expression in IM also suffers major alterations, including a marked increase in Le^a antigen expression [12, 23, 24, 25] which is thought to reflect the increased activity of FucT III enzyme [29]. López-Ferrer et al. [12] showed that the association between apomucins and Lewis antigens observed in the normal mucosa is lost in IM.

Our aim was to evaluate the mucins and type-1 Lewis antigens distribution pattern in IM and compare it with the "classic" types of IM defined using histochemistry. We used immunohistochemical techniques to detect the expression pattern of mucins (MUC1, MUC2, MUC5AC and MUC6) and carbohydrates (Le^a, Le^b, sialyl-Le^a and sulpho-$Le^{a/c}$) and histochemical techniques to classify IM types according to Filipe and Jass [6].

Materials and methods

Tissue samples and histochemistry

Endoscopic gastric biopsies (*n*=19) were obtained from asymptomatic volunteers or dyspeptic individuals from northern Portugal working in a shipyard at Viana do Castelo. Tissues were embedded in paraffin, and serial 4-μm sections were obtained for histochemistry and immunohistochemistry.

IM was classified according to Filipe and Jass [6], using alcian blue/periodic acid Schiff and high-iron diamine–alcian blue technique: in type-I (complete) IM the epithelium had mature absorptive non-secretory cells and goblet cells that secreted mainly sialomucins; in type-II (incomplete) IM the epithelium had few or absent absorptive cells, columnar mucous cells that secreted neutral mucins and/or small amounts of sialomucins and goblet cells that secreted sialo and occasionally sulphomucins; and in type-III (incomplete) IM columnar cells secreted predominantly sulphomucins and goblet cells produced sialo and/or sulphomucins.

Antibodies and immunohistochemistry

Monoclonal antibodies were used to detect mucins and type-1 chain carbohydrate antigens (Table 1). Paraffin sections were deparaffinated and rehydrated. Sections to be treated with the antibody against MUC2 (PMH1) were incubated with neuraminidase from type-VI *Clostridium perfringens* (Sigma Chemical Co. St. Louis, Mo.), diluted in 0.2 M sodium acetate buffer (pH 5.4) to a final concentration of 0.1 Units/ml, incubated for 2 h at 37°C and then washed two times in cold water and once in Tris-buffered saline (TBS; pH 7.6). In all sections endogenous peroxidase was blocked using 0.5% hydrogen peroxide (H_2O_2) in methanol for 30 min at room temperature. Sections were washed two times in TBS, incubated with rabbit normal serum, diluted 1:10 in bovine serum albumin (BSA) 10% for 20 min and incubated overnight at 4°C with the primary antibodies (Table 1).

Sections were rinsed, incubated for 30 min with the secondary antibody (biotin-labelled rabbit anti-mouse serum, diluted 1:200 in BSA 5%), rinsed in TBS and incubated with avidin–biotin–peroxidase complex for 1 h. Slides were washed three times in TBS before staining with 1 mg/ml 3.3′-diaminobenzidine tetrahydrochloride prepared in 0.05 M Tris–HCl containing 0.02% H_2O_2. Sections were then stained with haematoxylin, dehydrated and mounted in Entellan.

Table 1 Antibodies, specificity and references

Antibody	Specificity	Reference
HMFG1	MUC1	Taylor-Papadimitriou et al. 1981 [2]
PMH1	MUC2-GalNac	Reis et al. 1998 17]
CLH2	MUC5AC	Reis et al. 1997 [16]
CLH5	MUC6	Reis et al. 2000 [19]
Ca3F4	Lewis^a	Young et al. 1981 [28]
BG6	Lewis^b	Signet Pathology Systems
HB-80	Sialyl-Lewis^a	Magnani et al. 1982 [14]
F2	Sulpho-Lewis$^{a/c}$	Veerman et al. 1997 [26]

Table 2 Patterns of intestinal metaplasia (IM) defined using histochemistry

Number of biopsies	IM		
	Type I	Type II	Type III
8	+	+	–
6	+	–	–
3	–	+	–
2	+	+	+

Results

Characteristics of intestinal metaplasia classified using histochemistry

The results are summarised in Table 2. The 19 biopsies showed 16 foci of type-I IM with mature absorptive non-secretory cells and goblet cells that secreted sialomucins (Fig. 1h), 13 foci of type-II IM with columnar cells that secreted neutral mucins and/or small amounts of sialomucins and goblet cells that secreted sialo and occasionally sulphomucins (Fig. 2a, b) and two foci of type-III IM with columnar cells that secreted predominantly sulphomucins and goblet cells that secreted sialo and/or sulphomucins (Table 2).

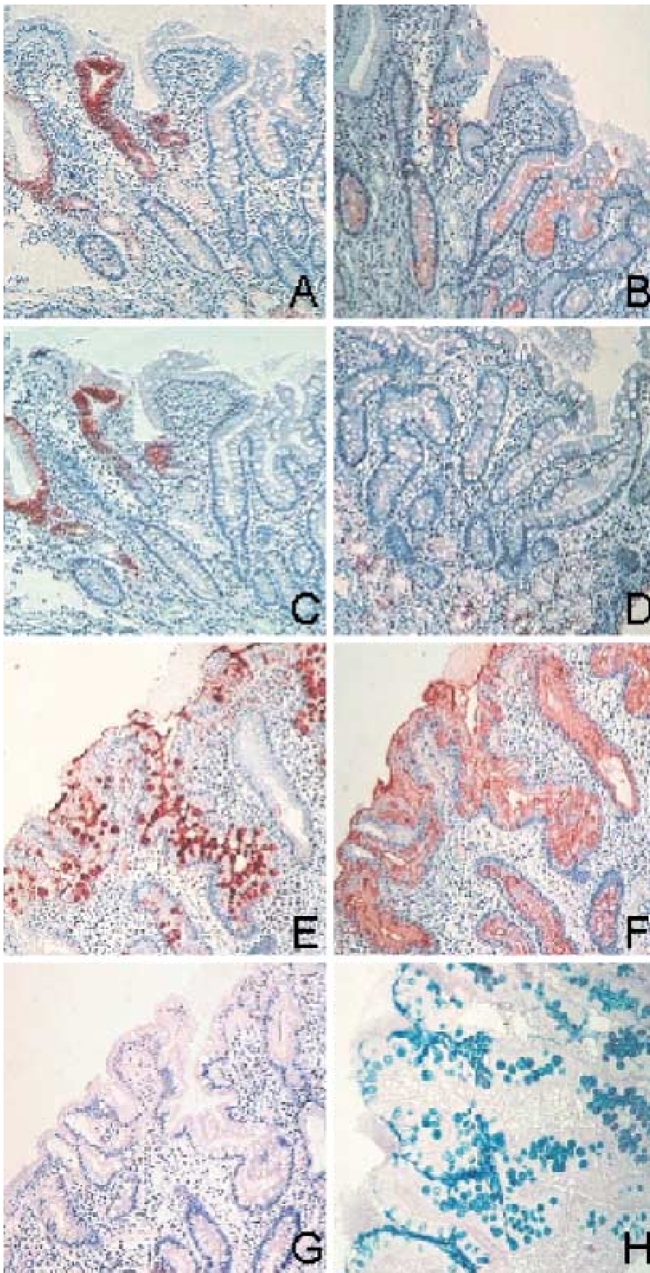

Fig. 1 Histochemical and immunohistochemical staining of serial sections from type-I intestinal metaplasia (IM). **a** IM does not express MUC1. **b** IM shows expression of MUC2 in goblet cells. **c** IM does not express MUC5AC. **d** IM does not express MUC6. **e** IM shows expression of Lewis type-1 chain antigen a (Le^a) in goblet cells. **f** IM shows expression of Lewis type-1 chain antigens b (Le^b) in goblet and columnar cells. **g** IM does not express $S\text{-}Le^a$. **h** Histochemical staining (high-iron diamine–alcian blue) of IM shows goblet cells stained in blue (sialomucins)

Characteristics of intestinal metaplasia classified using immunohistochemistry for MUC1, MUC2, MUC5AC and MUC6

All IM foci (n=31) showed MUC2 expression, in most cases (n=28) only in goblet cells. Four main patterns of mucin expression were observed in metaplastic tissue (Table 3). In 29.0% of the foci, expression of MUC2 was observed together with MUC1 and MUC5AC (Fig. 2c, d, e). In 25.8% of the foci, MUC2 was expressed together with MUC1. In 19.4% of the foci, only MUC2 was expressed (Fig. 1b). In 19.4% of the foci, the four mucins were expressed. One focus showed expression of MUC2 and MUC6, and one focus showed expression of MUC2 and MUC5AC (Table 3).

Table 3 Patterns of expression of mucins in intestinal metaplasia (IM)

Number of IM foci (%)	MUC1	MUC2	MUC5AC	MUC6
6 (19.4)	–	+	–	–
8 (25.8)	+	+	–	–
1 (3.2)	–	+	–	+
1 (3.2)	–	+	+	–
9 (29.0)	+	+	+	–
6 (19.4)	+	+	+	+

Table 4 Patterns of intestinal metaplasia (IM) defined using immunohistochemistry to type-1 Lewis antigens (Le)

Number of IM foci (%)	Le^a	Le^b	Sialyl-Le^a	Sulpho-$Le^{a/c}$
16 (55.2)	+	+	+	+
2 (6.9)	+	–	+	+
4 (13.8)	+	+	–	+
6 (20.7)	+	+	–	–
1 (3.4)	–	+	–	–

Characteristics of intestinal metaplasia classified using immunohistochemistry for type-1 Lewis antigens, Le^a, Le^b, sialyl-Le^a and sulpho-$Le^{a/c}$

Two cases of the present series were from individuals phenotypically Le^{b-} and were not used for this part of the study. The remaining cases showed 29 IM foci (Table 4). In 16 foci (55.2%) all type-1 Lewis antigens evaluated were expressed (Fig. 2g, h, i, j). Expression of Le^a, Le^b and sulpho-$Le^{a/c}$ was detected in 4 foci (13.8%). Le^a, sialyl-Le^a and sulpho-$Le^{a/c}$ were detected in two IM foci (6.9%). Le^a and Le^b were detected in six foci (20.7%), and there was one focus (3.4%) in which only Le^b was expressed.

Comparison between types of IM classified using histochemistry and patterns of immunohistochemical expression of mucins and type-1 chain carbohydrates

Serial sections were used to compare IM foci with the different histochemical and immunohistochemical stainings. The comparison was performed using as a reference system the classical classification of IM based on histochemistry. The results are summarised in Fig. 3.

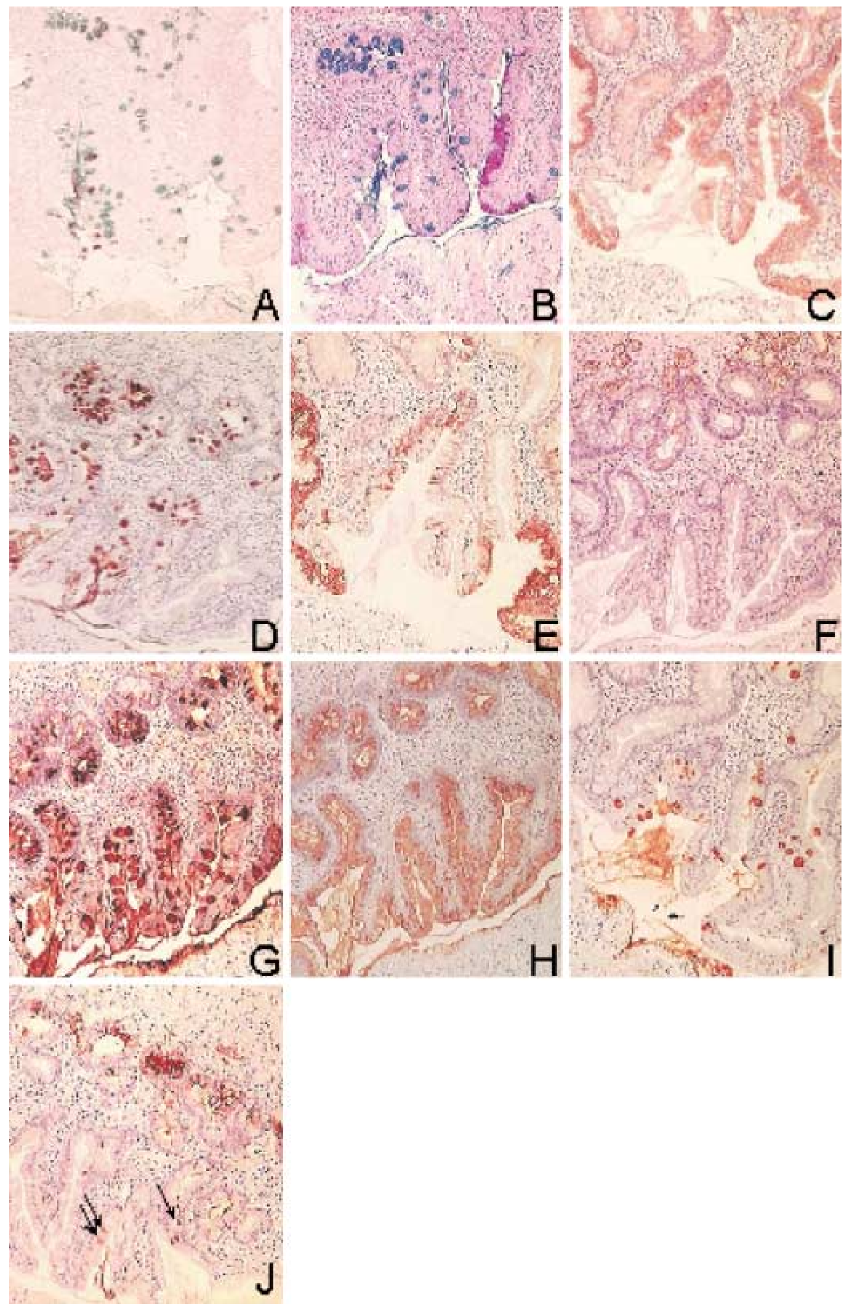

Fig. 2 Histochemical and immunohistochemical staining of serial sections of type-II intestinal metaplasia (IM). **a** Histochemical staining (high-iron diamine–alcian blue) of IM shows goblet cells stained in blue (sialomucins) and in brown (sulphomucins). **b** Histochemical staining (periodic acid Schiff) of IM shows columnar cells with a pink staining (neutral mucins). **c** Expression of MUC1 in goblet and columnar cells. **d** IM shows expression of MUC2 in goblet cells. **e** IM shows expression of MUC5AC in columnar cells. **f** IM does not express MUC6. **g** IM shows expression of Lewis type-1 chain antigen a (Le^a) in both goblet and columnar cells. **h** IM shows expression of Lewis type-1 chain antigen b (Le^b) in both goblet and columnar cells. **i** IM shows expression of Sialyl-Le^a in goblet cells. **j** IM shows expression of sulpho-Le^a in goblet cells (*arrows*)

All foci with type-I IM (n=16) expressed MUC2 in goblet cells. MUC1 was expressed in eight foci (50.0%) in columnar cells and in three of those also in goblet cells (Fig. 3). In most foci there was no expression of MUC5AC and MUC6, except in one focus that expressed MUC5AC in goblet cells and one focus that expressed MUC6 in columnar cells. In type-II IM (n=16) and type-III IM (n=2) there was expression of MUC1, MUC2 and MUC5AC in all foci studied. MUC1 and MUC5AC were expressed both in goblet and columnar cells, whereas MUC2 was predominantly expressed in goblet cells (Fig. 2c, d, e and Fig. 3). MUC6 was expressed in six foci (four in type-II IM and two in type-III IM) always in columnar cells.

As shown in Fig. 3 comparison between IM types regarding the expression of type-1 chain carbohydrates did not show major differences, except for an increased expression of Le^a in columnar cells of types-II and -III IM. In all IM types, sialyl-Le^a and sulpho-$Le^{a/c}$ were mostly expressed in goblet cells, although in some cases they were also present in columnar cells (Fig. 3).

The analysis of serial sections showed that there were nine foci of type-I IM and three foci of type-II IM that did not express sialyl-Le^a despite the presence of sialomucins disclosed using histochemistry. The presence of sulpho-$Le^{a/c}$ in goblet and columnar cells co-existed with the histochemical detection of sulphomucins in type-III IM. Sulpho-$Le^{a/c}$ was also detected in goblet and colum-

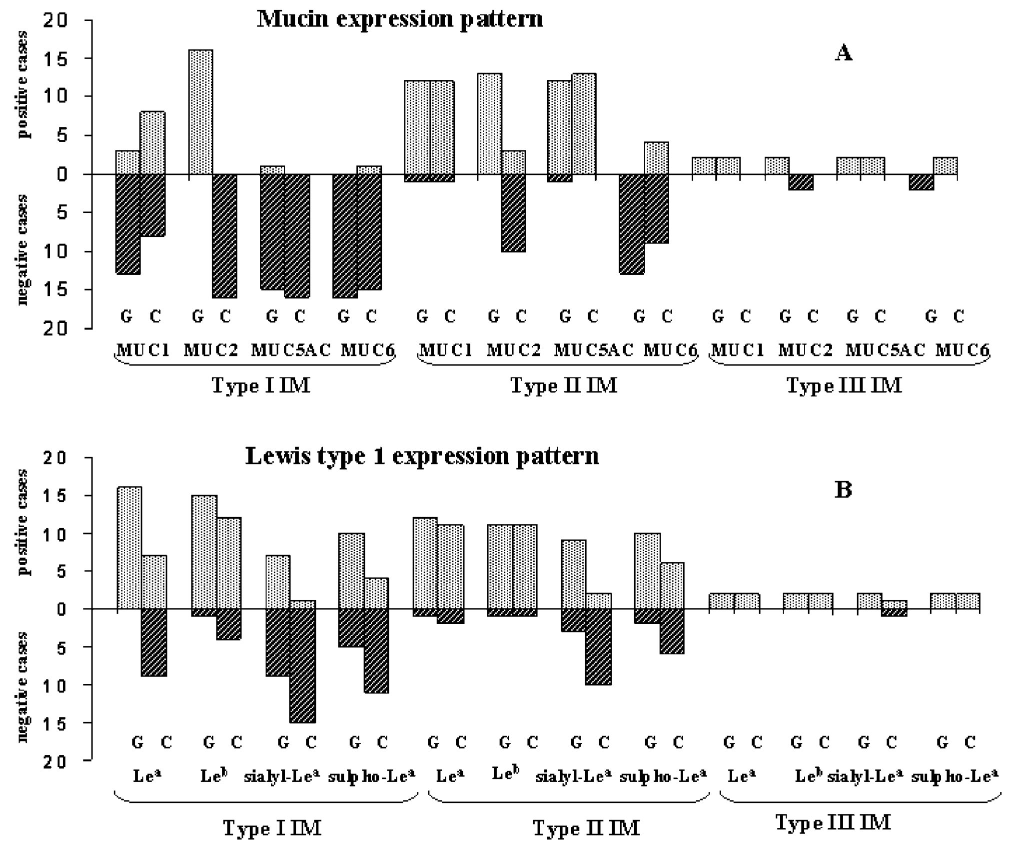

Fig. 3 Diagram representing mucin (**a**) and Lewis type-1 carbohydrate (**b**) expression pattern in intestinal metaplasia (IM). *G* goblet cells, *C* columnar cells. The three types of IM determined using histochemistry (I, II and III) were individualised, and for each type of IM the number of positive and negative cases for G and C cells was plotted according to mucin (**a**) and Lewis type-1 carbohydrate markers (**b**)

nar cells of type-I and type-II IM in the absence of histochemical detection of sulphomucins.

Discussion

We have shown that the mucin expression pattern of IM fits into two well-defined profiles: IM with expression of the intestinal mucin MUC2 and decreased/absent expression of MUC1, MUC5AC and MUC6 (complete IM); and IM with coexpression of the intestinal mucin MUC2 and the mucins usually expressed in the stomach – MUC1, MUC5AC and MUC6 (incomplete IM). These observations confirm previous results from our group both regarding the clear-cut difference between complete and incomplete IM and the similarity of the mucin expression profile in type-II and type-III IM [18]. The relatively higher frequency of type-I IM foci expressing MUC1 in the present series probably stems from a better fixation obtained in the biopsies evaluated in this study.

Lewis type-1 carbohydrate expression was identical in the different types of IM defined using histochemistry, except for the higher frequency of Le^a expression in columnar cells of types-II and -III IM. Anomalous expression of Le^a in IM was previously described by Torrado et al. [24]. In agreement with Torrado et al. [25], we observed that expression of Le^a in type-I IM was predominantly observed in goblet cells, whereas in types-II and -III IM, Le^a was observed both in goblet and in columnar cells. The aberrant expression of Le^a in IM is directly related to an increased expression of FucT III enzyme [29].

Sialyl-Le^a was mainly expressed in goblet cells in type-I IM (Fig. 3), in agreement with the histochemical observations showing sialylated mucin expression in goblet cells. However, in nine foci of type-I IM we did not detect expression of sialyl-Le^a, suggesting that other sialylated structures are expressed in this setting and are detected using histochemistry. One of the sialylated structures frequently expressed in IM is the sialyl-Tn antigen [3] which, in the present series, was expressed in most of the cases that were negative for sialyl-Le^a (data not shown).

In types-II and -III IM, sialyl-Le^a was expressed in goblet cells in most cases and in columnar cells in a smaller number of cases. The sialyl-Le^a expression pattern observed using immunohistochemistry showed partial overlap to the expression pattern of sialomucins determined using histochemistry. In three foci of type-II IM no expression of sialyl-Le^a was observed despite the presence of sialomucins in histochemical studies. Again, the aberrant expression of sialyl-Tn partially explains the observed discrepancies (data not shown).

In type-I IM, sulpho-Le$^{a/c}$ was expressed in goblet and columnar cells, although we did not detect the presence of sulphomucins in columnar cells using the high-iron diamine–alcian blue technique. These findings indicate that histochemistry has lower sensitivity than immunohistochemistry for identification of sulphomucins,

namely sulpho-Le$^{a/c}$. The same applies to the expression of sulpho-Le$^{a/c}$ that we observed in columnar cells of type-II IM foci showing only the presence of sialomucins by histochemistry.

The present study shows that the identification of complete versus incomplete IM is achieved using mucin immunohistochemistry as clearly as using classical histochemical methods using simpler and more reproducible techniques.

Despite the small size of the sample our results challenge the distinction of type-II from type-III IM. In fact, we did not observe any major difference between types-II and -III IM in the expression profile of both mucins and Lewis type-1 carbohydrates. Furthermore, we detected the presence of sulpho-Le$^{a/c}$ in columnar cells of all IM types regardless of the presence or absence of sulphomucins, thus weakening the basis for individualising type-III IM. The results observed in type-III IM in the present study were confirmed in ten cases of type-III IM obtained from gastric mucosa adjacent to gastric carcinomas in surgical specimens (David et al., unpublished observations).

Previous studies have shown the predictive value of identifying type-III IM in the risk assessment for gastric carcinoma development [7, 20]. Since we observed that immunohistochemical detection of sulpho-Le$^{a/c}$ is more "sensitive" than the histochemical detection of sulphomucins, future studies using large series classified using immunohistochemistry should be undertaken in order to evaluate the predictive value of sulpho-Le$^{a/c}$ expression in the context of all types of IM.

The two main conclusions of the present study for clinical and practical purposes are as follows: first, the present IM classification into complete and incomplete types should be performed using the immunohistochemical evaluation of mucin expression rather than histochemistry; second, for the evaluation of risk for gastric carcinoma development in patients with IM, future studies should take into account the expression of specific sulphated carbohydrates (e.g. sulpho-Le$^{a/c}$) rather than histochemical detection of sulphomucins.

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References

1. Biemer-Hüttmann AE, Walsh MD, McGuckin MA, Ajioka Y, Watanabe H, Leggett BA, Jass JR (1999) Immunohistochemical staining patterns of MUC1, MUC2, MUC4, and MUC5AC mucins in hyperplastic polyps, serrated adenomas, and traditional adenomas of the colorectum. J Histochem Cytochem 47:1039–1047
2. Carrato C, Balague C, de Bolós C, Gonzalez E, Gambus G, Planas J, Perini JM, Andreu D, Real FX (1994) Differential apomucin expression in normal and neoplastic human gastrointestinal tissues. Gastroenterology 107:160–172
3. David L, Nesland JM, Clausen H, Carneiro F, Sobrinho-Simões M (1992) Simple mucin-type carbohydrate antigens (Tn, Sialosyl-Tn and T) in gastric mucosa, carcinomas and metastases. Acta Pathol Microbiol Immunol Scand 27:162–172
4. De Bolós C, Garrido M, Real FX, (1995) MUC6 Apomucin shows a distinct normal tissue distribution that correlates with Lewis antigen expression in the human stomach. Gastroenterology 109:723–734
5. Filipe MI (1979) Mucins in the human gastrointestinal epithelium: a review. Invest Cell Pathol 2:195–216
6. Filipe MI, Jass JR (1986) Intestinal metaplasia subtypes and cancer risk. In: Filipe MI, Jass JR (eds) Gastric carcinoma. Churchill Livingstone, London, pp 87–115
7. Filipe MI, Munoz N, Matko I, Kato I, Pompe-Kirn V, Jutersek A, Teuchmann S, Benz M, Prijon T (1994) Intestinal metaplasia types and risk of gastric cancer: a cohort study in Slovenia. Int J Cancer 57:324–329
8. Gendler SJ, Spicer AP (1995) Epithelial mucin genes. Annu Rev Physiol 57:607–634
9. Guillem P, Billeret V, Buisine MP, Flejou JF, Lecomte-Houcke M, Degand P, Aubert JP, Triboulet JP, Porchet N (2000) Mucin gene expression and cell differentiation in human normal, premalignant and malignant esophagus. Int J Cancer 88:856–861
10. Ho SB, Shekels LL, Toribara NW, Kim YS, Lyftogt C, Cherwitz DL, Niehans GA (1995) Mucin gene expression in normal, preneoplastic, and neoplastic human gastric epithelium. Cancer Res 55:2681–2690
11. Lapensee L, Paquette Y, Bleau G (1997) Allelic polymorphism and chromosomal localization of the human oviductin gene (MUC9). Fertil Steril 68:702–708
12. López-Ferrer A, de Bolós C, Barranco C, Garrido M, Isern J, Carlstedt I, Reis CA, Torrado J, Real FX (2000) Role of fucosyltransferases in the association between apomucin and Lewis antigen expression in normal and malignant gastric epithelium. Gut 47:349–356
13. López-Ferrer A, Curull V, Barranco C, Garrido M, Lloreta J, Real FX, de Bolós C (2001) Mucin as differentiation markers in bronchial epithelium – Squamous cell carcinoma and adenocarcinoma display similar expression patterns. Am J Respir Cell Mol Biol 24:22–29
14. Magnani JL, Nilsson B, Brockhaus M, Zopf D, Steplewski Z, Koprowski H, Ginsburg V (1982) A monoclonal antibody-defined antigen associated with gastrointestinal cancer is a ganglioside containing sialylated lacto-N-fucopentose II. J Biol Chem 257:14365–14369
15. Mollicone R, Bara J, Le Pendu J, Oriol R (1985) Immunohistologic pattern of type 1 (Le^a, Le^b) and type 2 (X, Y, H) blood group-related antigens in the human pyloric and duodenal mucosae. Lab Invest 53:219–227
16. Reis CA, David L, Nielsen PA, Clausen H, Mirgorodskaya K, Roepstorff P, Sobrinho-Simões M (1997) Immunohistochemical study of MUC5AC expression in human gastric carcinomas using a novel monoclonal antibody. Int J Cancer 74:112–121
17. Reis CA, Sorensen T, Mandel U, David L, Mirgorodskaya E, Roepstorff P, Kihlberg J, Stig-Hansen JE, Clausen H (1998) Development and characterization of an antibody to an α-*N*-acetil-D-galactosamine glycosylated MUC2 peptide. Glycoconj J 15:51–62
18. Reis CA, David L, Correa P, Carneiro F, de Bolós C, Garcia E, Mandel U, Clausen H, Sobrinho-Simões M (1999) Intestinal metaplasia of human stomach displays distinct patterns of mucin (MUC1, MUC2, MUC5AC, and MUC6) expression. Cancer Res 59:1003–1007
19. Reis CA, David L, Carvalho F, Mandel U, de Bolós C, Mirgorodskaya E, Clausen H, Sobrinho-Simões M (2000) Immunohistochemical study of the expression of MUC6 mucin and co-expression of other secreted mucins (MUC5AC and MUC2) in human gastric carcinomas. J Histochem Cytochem 48:377–388
20. Rokkas T, Filipe MI, Sladen GE (1991) Detection of an increased incidence of early cancer in patients with intestinal metaplasia type III who are closely followed up. Gut 32:1110–1113
21. Shankar V, Pichan P, Eddy RL Jr, Tonk V, Nowak N, Sait SN, Shows TB, Schultz RE, Gotway G, Elkins RC, Gilmore MS, Sachdev GP (1997) Chromosomal localization of a human mucin gene (MUC8) and cloning of the cDNA corresponding to the carboxy terminus. Am J Respir Cell Mol Biol 16:232–241

22. Taylor-Papadimitriou J, Peterson JA, Arklie J, Burchell J, Ceriani RL, Bodmer WF (1981) Monoclonal antibodies to epithelium-specific components of the human milk fat globule membrane: production and reaction with cells in culture. Int J Cancer 28:17–21
23. Torrado J, Blasco E, Cosme A, Gutierrez-Hoyos A, Arenas JI, (1989) Expression of type 1 and type 2 blood group-related antigens in normal and neoplastic gastric mucosa. Am J Clin Pathol 91:249–254
24. Torrado J, Blasco E, Gutierrez-Hoyos A, Cosme A, Lojendio M, Arenas JI (1990) Lewis system alterations in gastric carcinogenesis. Cancer 66:1769–1774
25. Torrado J, Plummer M, Vivas J, Garay J, Lopez G, Peraza S, Carillo E, Oliver W, Muñoz N (2000) Lewis antigen alterations in a population at high risk of stomach cancer. Cancer Epidemiol Biomarkers Prev 9:671–674
26. Veerman ECI, Bolscher JGM, Appelmelk BJ, Bloemena E, van den Berg TK, Nieuw Amerongen AV (1997) A monoclonal antibody directed against high M_r salivary mucins recognizes the SO_3-3Galβ1–3GlcNAc moiety of sulfo-Lewis[a]: a histochemical survey of human and rat tissue. Glycobiology 7:37–43
27. Williams SJ, McGuckin MA, Gotley DC, Eyre HJ, Sutherland GR, Antalis TM (1999) Two novel mucin genes down-regulated in colorectal cancer identified by differential display. Cancer Res 59:4083–4089
28. Young WW Jr, Portoukalian J, Hakomori S (1981) Two monoclonal anticarbohydrate antibodies directed to glycosphingolipids with a lacto-N-glycosyl type II chain. J Biol Chem 256:10967–10972
29. Yuzuro I, Nishihara S, Kudo T, Higara T, Morozumi K, Hattori T, Narimatsu H (1998) The aberrant expression of Lewis[a] antigen in intestinal metaplastic cells of gastric mucosa is caused by augmentation of Lewis enzyme expression. Glycoconj J 15:799–807