Monoclonal antibodies reacting with the MUC2 mucin core protein

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Summary This study sought to produce monoclonal antibodies (MAbs) which reacted with the MUC2 core protein. Two MAbs [3A2 (IgG1) and 4F1 (IgM)] were produced by immunising female BALB/c mice with gel-formed mucin from the LS174T colon cancer cell line followed by a KLH conjugate of a 29 amino acid synthetic peptide whose sequence was derived from the variable number of tandem repeats (VNTR) region of a MUC2 cDNA clone. The MAbs reacted with synthetic MUC2 VNTR peptides but not synthetic MUC1 or MUC3 VNTR peptides, and showed specific reactivity in Western blotting with a high molecular weight protein produced by the LS174T colon carcinoma cell line. The use of shorter peptides indicated that the minimum peptide epitopes for these MAbs were different. Mab 3A2 reacted with amino acids 5–19 of the MUC2 VNTR by inhibition ELISA but not by direct ELISA, while 4F1 reacted with this peptide in both assays. Furthermore, 4F1 reacted in direct ELISA when a larger (29 amino acid) MUC2-derived peptide was coated onto the assay plate by incubating in carbonate buffer or by drying the peptide onto the assay plate, while 3A2 only reacted when this peptide was coated in carbonate buffer. The different specificity of the MAbs was also illustrated by the reactivity of 4F1 but not 3A2 with partially deglycosylated cystic fibrosis mucin.

Immunohistochemical analysis with these MAbs revealed a strong reaction with lung, gastric and colon tumours relative to normal tissue, with some breast and ovarian tumours also reacting. Both MAbs stained some normal goblet cells in the perinuclear region but not the mucin droplet or secreted mucin, indicating a reaction with immature (poorly glycosylated) mucin in the endoplasmic reticulum and/or golgi, but not with mature (fully glycosylated) mucin. In contrast, tumours showed strong diffuse cytoplasmic staining. 4F1 also showed weak apical cytoplasmic staining in some goblet cells and stained some tumours which showed no reactivity with 3A2.

These antibodies should prove useful in the study of MUC2 structure and function, and in the diagnosis of some tumours.

Mucins are a family of highly glycosylated, high molecular weight (>200 kDa) glycoproteins present on the surface of many epithelial cells (Devine & McKenzie, 1992). Increased expression of mucin epitopes on tumour cells makes them suitable candidates as tumour markers. Five distinct gene loci have now been identified in humans, these being renamed MUC1, MUC2 and MUC3 (Human Gene Mapping Nomenclature Committee, 1989) and the names MUC4 and MUC5 have been proposed (Porchet et al., 1991; Aubert et al., 1991). Each gene codes for a protein containing a variable number of tandem repeats (VNTR) of 20 (MUC1), 23 (MUC2), 17 (MUC3), 16 (MUC4), and 8 (MUC5) amino acids, but there is no significant homology between the different VNTRs (Gendler et al., 1987; Gum et al., 1989; 1990; Porchet et al., 1991; Aubert et al., 1991). Many monoclonal antibodies (MAbs) reacting with the MUC1 VNTR have been reported (Gendler et al., 1988; Xing et al., 1990; Layton et al., 1990; Price et al., 1990), and assays incorporating some of these MAbs have been shown to be particularly useful in monitoring patients with breast and ovarian cancer (Ward et al., 1993; Safi et al., 1991; Bhargava et al., 1989). As well as overexpression of MUC1, altered glycosylation of the VNTR is responsible for the exposure of these peptide epitopes in tumours (Gendler et al., 1988; Devine et al., 1990a).

Despite the success of MUC1 VNTR-reactive MAbs, only a few MAbs reacting with other VNTRs have been reported (Xing et al., 1992; Price et al., 1991), and the use of these MAbs for diagnosis and therapy has not been investigated. The MUC2 mucin is of particular interest since this is a major component of mucus produced by patients with colon and lung cancer, as well as those with cystic fibrosis (Gum et al., 1989; Gerard et al., 1990; Jany et al., 1991). Subsequently, by immunising with native colon cancer mucin and a KLH-synthetic peptide conjugate containing the 23 amino acid MUC2 VNTR sequence, we have produced anti-MUC2 VNTR MAbs which react with the intact mucin. These MAbs show a high reactivity with colon, gastric, and lung tumours by immunohistochemistry, and may prove useful in the diagnosis and therapy of these tumours.

Materials and methods

Peptides

The peptides used in this study are shown in Table I. The M1, M2 and T4N1 peptides were synthesised using an Applied Biosystems Model 430A automated peptide synthesiser (Forster City, CA, USA) by Merrifield solid phase synthesis (Hodges & Merrifield, 1975). M1 corresponds to the 20 amino acid MUC1 repeat plus the first four amino acids of the next repeat (Gendler et al., 1987); M2 corresponds to the first 23 amino acid MUC2 repeat plus the first four amino acids of the next repeat (Gum et al., 1989), with KY added to the N-terminal for conjugation; T4N1 corresponds to the N-terminus of mouse CD4 (Clark et al., 1988), and was used as a control. The M2a, v peptide, synthesised on 'pins' (Gysen et al., 1984), was donated by Chiron Mimotopes, Australia, and corresponded to amino acids 5–19 of the first MUC2 repeat (Gum et al., 1989). The M2a and M2c peptides were produced by cyanogen bromide cleavage of the M2 peptide (Gross, 1967), and correspond to the N-terminal and C-terminal portions of the M2 peptide. The M3 peptide was prepared using the 'tea bag' method (Houghton, 1985), and represents the 17 amino acid MUC3 repeat plus the first five amino acids of the next repeat (Gum et al., 1990), with lysine attached to the N-terminus for conjugation. The pep-
tides were purified by reversed-phase HPLC on a Deltapak-C18 column (Nihon Waters Ltd, Tokyo, Japan), with a gradient of acetonitrile in 0.1% TFA, and the identity of each was confirmed by N-terminal sequencing and mass spectrometry. The M2 peptide was conjugated to keyhole limpet hemocyanin (KLH) using glutaraldehyde (Zegers et al., 1990).

### Mucins

The LS174T and HT29-SB colon cancer cell lines have been shown to secrete significant quantities of mucin (Devine et al., 1991, 1992). Much of the LS174T and HT29-SB mucin is secreted into the culture media as a viscoelastic gel, which was harvested by filtration on double thickness lens tissue, and washed twice on the tissue with water. Cytic fibrosis (CF) mucin, donated by Dr G. Sachdev, was purified and deglycosylated with trifluoromethane sulphonic acid (TFM-SA) (Desai et al., 1991). Human milk fat globule membranes (HMFGM) were used as a source of MUC1 mucin (Devine et al., 1990b).

### Production of monoclonal antibodies

BALB/c female mice (8 weeks old) were injected by s.c. and i.m. routes with LS174T mucin emulsified in Freund’s Complete Adjuvant. A second injection was given 6 weeks later, except the mucin was emulsified in Freund’s Incomplete Adjuvant (FIA) and injected by i.p. and i.m. routes. After a further 6 weeks, the mouse was injected i.p. and i.m. with M2-KLH conjugate in FIA. Four weeks later, M2-KLH was given i.v. in PBS and i.p. in FIA, and the i.p. injection was repeated for the next 3 days, as this protocol had been shown to give greater success in the production of anti-peptide MAbs (Schibier et al., 1988). The spleen cells were fused with NS1 cells the next day.

Hybridomas were screened by ELISA on LS174T mucin, M2 and T4N1 peptides (Layton et al., 1990). Positive clones were then checked by ELISA on M1, and by immunoblotting on LS174T and HT29-SB mucins (Devine & Birrell, 1992). Those showing specificity for LS174T and M2 were cloned by limiting dilution. The subclass of MAbs was determined by dual-determinant ELISA using subclass-specific antibodies to capture MAbs and anti-mouse Ig-peroxidase (Silenus, Australia) to detect bound MAb.

### ELISA

All assays were performed in duplicate, with the percentage coefficient of variation of duplicates being <10% in all cases. MAbs BC2 (IgG1), BC3 (IgM), 401/21 (IgG1), and FM1 (IgM) were used as control antibodies (Xing et al., 1989; Skerritt & Hill, 1990; Devine et al., 1990b). BC2 and BC3 react with the minimum epitope APDTR on the MUC1 VNTR (Xing et al., 1990), 401/21 reacts with wheat protein gliadins (Skerritt & Hill, 1990), while the specificity of FM1 has not been determined.

### Solid-Phase ELISA

Peptides (2.5 µg dry weight ml⁻¹) or mucins (40 µg dry weight LS174T mucin ml⁻¹ or 5 µg dry weight CF mucin ml⁻¹) were coated onto a Falcon flexible

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**Table 1** Peptide inhibition of 3A2 and 4F1 binding

| Peptide | Sequence | MW (kDa) | % Inhibition of 3A2 | % Inhibition of 4F1 |
|---------|----------|----------|---------------------|---------------------|
| M1      | PDTRPAGSTAPPAGHVTSSAPDTR | 2359.1 | nd | nd |
| M2      | KYPTTPSTTTIMTVTPTPTGQTPTTTTT | 3023.7 | 99 | 97 |
| M2a,19  | PISITTMVVTPTPTPT | 1512.8 | 100 | 98 |
| M2a     | KYPTTPSTTTTM | 1441.7 | 33 | 66 |
| M2c     | VTPPTPTPTGQTPTTTTT | 1600.8 | 90 | 93 |
| M3      | KSHSTPSFTSSTTSTSHSTTP | 2422.6 | 5 | 9 |
| T4N1    | KTLYLGKEQESAELPCECY | 2158.6 | 0 | 0 |

*nd, not done.*
representative of the tumour was selected; and 5 μm sections were mounted on glass slides for immunohistochemical analysis. Tissue tested comprised; ten cases each of colonic, gastric, and lung cancers (five adenocarcinomas, two squamous cell carcinomas, one adenosquamous, one epidermoid) and 11 breast cancers; seven cases of non-mucinous and seven cases of mucinous cystadenocarcinomas of the ovary; and five cases of benign ovarian tumours. Stained sections were analysed by a single investigator (MAM) and the antigen was recorded as being either absent or present in less than 25%, 25–50%, 50–75%, or more than 75% of tumour cells. The cellular localisation of antigens was recorded as being membranous, cytoplasmic, or both (membrane staining was defined as being luminal alone or along the entire membrane). Intensity of staining was scored on a four point scale. Non-malignant colonic, gastric, lung and breast tissue, obtained from non-involved resection margins were also assessed and expression of the antigens described. Mesothelioma cells were isolated from pleural fluid and treated as above, except fixation was done in methacarn.

Immunohistochemistry techniques
Immunohistochemistry was performed (McGuckin et al., 1990) with MAb ascites at 1000⁻¹. Sections were stained with 3A2, 4F1, and negative control MAb FM1.

Results

Production of MUC2 reactive monoclonal antibodies
Monoclonal antibodies 3A2 (IgG), and 4F1 (IgM) were chosen after the fusion, as these reacted with LS174T mucin and the M2 peptide, but not HMFGM or the M1, M2 and T4N1 peptides (not shown). Control MAbs 401/21 and FM1 were negative in all cases, while MAbs BC2 and BC3 reacted with HMFGM and the M1 peptide but not with other antigens (not shown). After these hybridomas were cloned and produced as ascites in mice, checkerboard titration showed that the reactivity of both MAbs with M2 peptide and LS174T mucin was concentration dependent (Figure 1).

Reactivity of antibodies with peptides
The results of inhibition ELISA with different peptides is shown in Table I. The binding of both MAbs to the M2 peptide was inhibited by M2, M2₅₋₁₉, M2₂₀, and M2₆ peptides, but not the M3 or T4N1 peptides (M1 not tested). However, the M2, M2₅₋₁₉, and M2₆ peptides showed greater inhibition of MAb binding than the M2₆ peptide, particularly with 3A2. It is also of interest to note that MAb 4F1 also reacted with the M2₅₋₁₉ peptide by solid-phase ELISA while MAb 3A2 showed no reactivity with this peptide in this assay system. Furthermore, when peptides were dried in vacuo on to the microtitre plate, 3A2 showed no reactivity with M2 while 4F1 reacted strongly (not shown).

Reactivity of antibodies with cystic fibrosis (CF) mucin
The reactivity of MAbs with native and partially deglycosylated cystic fibrosis mucin was determined by solid-phase ELISA. MAb 3A2 showed very weak reactivity with either mucin (not shown), while 4F1 reacted weakly with native mucin and showed strong reactivity with partially deglycosylated CF mucin produced by TFMSA treatment (Figure 2). Control MAbs 401/21 and FM1 were negative.

Reaction of 3A2 and 4F1 with human tumour cell lines
A panel of human colon cancer cell lines was tested in the cell ELISA with MAbs 3A2 and 4F1 (Table II). Staining ranged from 0–100% of cells, with cytoplasmic staining in all cases. The LIM2463 cell line showed the greatest expression of the peptide epitopes detected by 3A2 and 4F1 (100%...
of cells reactive), while the LIM1899 cell line also showed strong reactivity in 25% of cells. The MAbs also reacted with the LS174T cell line, which was the source of the MUC2 used as immunogen.

Analysis of mucins by western blotting
Gel-formed mucin from the LS174T colon carcinoma cell line reacted with MAbs 3A2 and 4F1, but not control MAbs 401/21 or FM1 (Figure 3). Both MAbs reacted under reducing and non-reducing conditions with a single high molecular weight band of molecular weight greater than 400 kDa. There was no reaction, however, with gel-formed mucin produced by the HT29-SB colon carcinoma cell line (not shown).

Reactivity of antibodies with non-malignant tissue by immunoperoxidase staining
Both 4F1 and 3A2 antibodies reacted with some epithelial components of non-malignant colonic, gastric and lung tissue, but not with non-malignant breast epithelium. In normal colon, 4F1 antigen expression was typified by diffuse cytoplasmic staining of the colonic mucosal surface, although antigen was not detected within goblet cell mucin droplets. The proportion of positive cells varied between specimens from 10 to 75% of surface epithelial cells, and staining decreased deeper in the mucosal crypts. Occasionally, stronger granular staining in peri- and supra-nuclear regions of goblet cells was observed (Figure 4a). In contrast, 3A2 expression was mainly restricted to such granular staining in the basal region of goblet cells, although some specimens showed a small proportion of cells with diffuse cytoplasmic staining similar to that found for 4F1. In most specimens, less than 10% of goblet cells were positive with most staining in the outer cells of crypts (Figure 4b,c). In normal stomach, 4F1 staining revealed diffuse cytoplasmic antigen in pyloric glands and more granular cytoplasmic staining in cells of the surface epithelium. The proportion of cells positive varied widely between specimens from no staining to about 25% of cells positive. 3A2 expression was less but similar where present, although in some specimens surface epithelial cells showed strong granular cytoplasmic staining confined to the basal region of the cell. In lung tissue, neither 4F1 or 3A2 stained alveoli but some weak staining was observed with both antibodies in bronchioles and mucinous glands. Both bronchial epithelium diffuse cytoplasmic staining was found in the apical region of some columnar cells but staining was not observed in mucin droplets of goblet cells. In mucinous glands diffuse cytoplasmic staining was found in a small proportion of cells using 4F1 but not 3A2. Only one of five benign ovarian tumours was positive for 4F1, and none for 3A2. The 4F1 positive tumour was a benign mucinous tumour that showed cytoplasmic staining in almost all cells.

Reactivity of antibodies with malignant tumours by immunoperoxidase staining
Expression of the epitopes defined by 4F1 and 3A2 in malignant tumours are summarised in Table III, with representative sections shown in Figure 4. Expression of each antigen was variable within all tumour types. All colon carcinomas were positive for both 4F1 and 3A2 with the exception of one well differentiated carcinoma of the sigmoid colon. Although typically less cells were positive for 3A2 than for 4F1, 3A2 often revealed strong granular cytoplasmic staining compared with the diffuse cytoplasmic staining characteristic of 4F1. Granular cytoplasmic staining was not restricted to a subcellular compartment as was the case in non-malignant epithelium. Neither membrane or extracellular antigen was detected by either antibody. Most gastric carcinomas showed cytoplasmic 4F1 expression in a majority of tumour cells. Less tumours were positive for 3A2, and where the antigen was present a lower proportion of tumour cells were positive than for 4F1. Strong granular staining was again more typical of 3A2 than 4F1. All lung cancers were positive for the 4F1 antigen with the exception of the one neuroendocrine tumour. Six of ten lung tumours showed 3A2 expression, and where the antigen was present a lower proportion of tumour cells were positive. Expression of both antigens was usually of weak to moderate intensity with diffuse cytoplasmic staining, although in some tumours foci of coexistent strong granular cytoplasmic staining were present. The 4F1 epitope was found in both mucinous and non-mucinous ovarian carcinomas, although the proportion of cells positive was greater in mucinous tumours. Staining of ovarian tumours was also restricted to the cytoplasm. Only half of the ovarian tumours were positive for 3A2 and where present this epitope was typically restricted to a small proportion of tumour cells. The 4F1 and 3A2 epitopes were found in approximately half of the cases of invasive breast carcinoma. Staining was cytoplasmic except for weak coexistent membrane staining in two of the cases. Ductal carcinoma in situ was present in two of the positive cases, and in both cases, these lesions expressed the 4F1 and 3A2 epitopes, although with a different staining pattern to the adjacent invasive tumour. The antibodies also reacted with 5/6 (4F1) and 3/6 (3A2) mesotheliomas.

Discussion
The isolation of cDNA clones coding for the protein core of MUC2 mucin (Gum et al., 1990) has enabled us to use a synthetic peptide as immunogen for the production of anti-MUC2 core peptide-reactive monoclonal antibodies. Two MAbs were produced, 3A2 (IgG1) and 4F1 (IgM), and these reacted specifically with synthetic MUC2-derived peptides, colon carcinoma cell lines, and paraffin embedded sections of various cancer tissue. However, subtle differences in the reactivity of the two MAbs were observed.

Both MAbs reacted with MUC2 peptide but not MUC1 or MUC3 peptides, and showed specific reactivity with a high
molecular weight mucin-like molecule produced by the LS174T colon carcinoma cell line. It was of interest that these MAbs showed some reactivity with the shorter MUC2 peptides tested, which represent different regions of the MUC2 VNTR. All peptides (M2e, M2n, and M2c) contained the sequence TTT, as well as PT and TP containing regions, suggesting that these amino acids may be part of the epitopes for these MAbs. The M3 peptide, which was not detected by these MAbs, also contains the sequence TTT, suggesting that TTT is not the minimum epitope. Furthermore, the M2n peptide showed weaker inhibition than the other peptides, suggesting that the epitope for optimum binding may lie in the region TPTPTP common to the M2e, M2n, and M2c peptides. It is of interest that MAb GL-013, raised against gastric carcinoma cells, was shown to react with a TTT-containing minimum epitope on the MUC2 VNTR (Price
Table III  Expression of the antigens defined by monoclonal antibodies 4F1 and 3A2 in malignant tumours of the colon, stomach, lung, ovary, and breast, and mesotheliomas

| Tumour type | Percentage of tumour cells positive* | n  | 0 | 1–25 | 25–50 | 50–75 | 75–100 |
|-------------|-----------------------------------|----|---|------|------|------|------|
| 4F1         |                                   |    |   |      |      |      |      |
| Colon       |                                   | 10 | 1 | 2    | 0    | 4    | 3    |
| Stomach     |                                   | 10 | 1 | 1    | 0    | 2    | 6    |
| Lung        |                                   | 10 | 1 | 1    | 2    | 2    | 4    |
| Ovary muc   |                                   | 7  | 1 | 1    | 1    | 2    | 2    |
| non-muc     |                                   | 7  | 2 | 2    | 1    | 7    | 10   |
| Breast      |                                   | 11 | 1 | 1    | 1    | 1    | 1    |
| Mesothelioma|                                   | 6  | 1 | 1    | 0    | 0    | 5    |
| 3A2         |                                   |    |   |      |      |      |      |
| Colon       |                                   | 10 | 1 | 5    | 2    | 1    | 1    |
| Stomach     |                                   | 10 | 3 | 3    | 2    | 2    | 0    |
| Lung        |                                   | 10 | 4 | 2    | 3    | 0    | 1    |
| Ovary muc   |                                   | 7  | 3 | 2    | 1    | 1    | 0    |
| non-muc     |                                   | 7  | 4 | 2    | 1    | 0    | 0    |
| Breast      |                                   | 11 | 6 | 2    | 2    | 0    | 0    |
| Mesothelioma|                                   | 6  | 3 | 0    | 0    | 1    | 2    |

*Control mab FM1 was negative on all samples.

et al., 1991), suggesting that 3A2 and 4F1 react with a different part of the MUC2 VNTR. However, the position of an epitope in a synthetic peptide has been shown to affect mAb reactivity (McKenzie & Xing, 1990), and firm conclusions regarding the epitopes for these MAbS cannot be drawn from these experiments. Further experiments with shorter overlapping peptides are needed to define the minimum epitopes of these MAbS.

Differences in the fine specificity of MAbS 3A2 and 4F1 were also illustrated by the reactivity of 3A2 with the M2,19 peptide by inhibition ELISA but not direct ELISA, while 4F1 reacted with this peptide in both assay systems. In addition, the reactivity of 3A2 with the M2 peptide in direct ELISA was dependent on the method of plate coating. This may explain the weaker reactivity of 3A2 with LS174T mucin and CF mucin by direct ELISA. In addition, the consensus sequence of the MUC2 VNTR from intestine is slightly different to that of tracheobronchial tissue (Gerard et al., 1991), so the difference in reactivity with CF mucin may be due to differences in the minimum epitopes of 3A2 and 4F1.

The observations from immunohistochemical staining of both normal and malignant tissues are consistent with 4F1 and 3A2 recognising different epitopes on the protein core of the MUC2 mucin. Cellular distribution of these epitopes in normal gastro-intestinal and respiratory tissues was similar to that described for antigens recognised by polyclonal antibodies prepared against deglycosylated LS174T mucin (Yan et al., 1990) and deglycosylated sputum from a cystic fibrosis patient (Perini et al., 1989). The perinuclear granular staining, seen particularly with 3A2, probably represents detection of the protein core in the endoplasmic reticulum or golgi apparatus prior to the completion of glycosylation. The lack of reactivity of both antibodies with mucin droplets of goblet cells and luminal secretions suggests low reactivity with the mature mucin in normal cells. The observation that not all cells of a given type showed equal expression of either epitope is consistent with the findings of other studies utilising various antibodies to colonic and/or respiratory mucins (Yan et al., 1990; Perini et al., 1989; Podolsky et al., 1986; Finkbeiner & Basbaum, 1988). These findings suggest either phenotypic or stage dependent maturational differences may exist in mucin production patterns of morphologically similar cells.

Detection of the 4F1 and 3A2 epitopes in colon, gastric, lung, breast, and ovarian cancers demonstrates continued MUC2 production by at least some carcinomas derived from these organs. The expression of both epitopes in some malignancies was much greater than their expression by corresponding non-malignant cells. Increased expression in cancers could reflect either increased production and/or underglycosylation of MUC2. The loss of compartmentalisation of granular cytoplasmic staining is consistent with the loss of polarity that occurs following malignant transformation of epithelial cells. Larger numbers of specimens are required to fully assess the relative expression in different histological types. However, it appears the degree of differentiation has little effect on expression in colon and gastric carcinomas. MUC2 was expressed in both mucinous and non-mucinous ovarian carcinomas. Unfortunately, this finding prevents the use of MUC2 detection for differential diagnosis of gastrointestinal and mucinous ovarian pelvic and peritoneal malignancies. In addition, MUC2 was also expressed by mesotheliomas, and these MAbS cannot be used for the differential diagnosis of mesothelioma and adenocarcinoma. The 4F1 and 3A2 epitope expression detected in some cases of breast carcinoma is consistent with the findings of Yan et al. (1990) who found a small percentage of cells positive for antibodies against deglycosylated colon mucin in one of five breast cancers, and Jany et al. (1991) who demonstrated the presence of MUC2 RNA in mammary tissue. Although antigen was not detected in normal breast duct epithelium, further analysis is required as MUC2 may be expressed by foetal or lactating breast epithelium.

These antibodies should prove useful in the study of MUC2 structure and function, and also have potential as diagnostic and therapeutic agents. The production of second generation anti-peptide antibodies represents a useful method of producing anti-tumour reagents, and this technology may also be applied to the production of MAbS to the other mucin core proteins.

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References

AUBERT, J.-P., PORCHET, N., CREPIN, M., DUTERQUE-COQUIL-LAUD, M., VERGNES, G., MAZZUCA, M., DEBUJRE, B., PETITREZ, D. & DEGAND, P. (1991). Evidence for different human tracheobronchial mucin peptides deduced from nucleotide cDNA sequences. Am. J. Resp. Cell. Biol. Med., 5, 178–185.

BHARGAVA, A.K., PETRELLI, N.J., MYERS, M.M., KHAN, S., FITZ-PATRICK, J., BURKE, P., ANSELMINO, L. & MANDERINO, G. (1989). A new breast cancer marker (BCM) in monitoring disease status. J. Tumour Marker Oncol., 4, 373–381.

CLARK, G.J., TOBIAS, G.H., PIETERSZ, G.A., CLASSEN, B.J., WALKER, I.D., MCKENZIE, I.F.C. & DEACON, L.J. (1988). Isolation of a cDNA clone from the murine CD4 antigen. Transplant. Proc., 20, 45–48.

DESAI, V.C., NAZIRUDDIN, B., GRAVES, D.C., DE LA ROCHA, S.R. & SACHDEV, G.P. (1991). Production and characterisation of monoclonal antibodies to purified deglycosylated cystic fibrosis respiratory mucin: evidence for the presence of four immunologically distinct epitopes. Hybridoma, 10, 285–296.

DELINE, P.L., WARD, B.G., MCKENZIE, I.F.C. & LAYTON, G.T. (1990a). Glycosylation and the exposure of tumor-associated epitopes on mucins. J. Tumor Marker Oncol., 5, 11–26.

DELINE, P.L., WARD, J.A., CLARK, B.A., LAYTON, G.T., WARD, B.G., MACDONALD, B., XING, P.-X. & MCKENZIE, I.F.C. (1990b). The complexity of cancer-associated epitope expression on antigens produced by ovarian tumor cells. J. Tumor Marker Oncol., 5, 221–339.

DELINE, P.L., LAYTON, G.T., CLARK, B.A., BIRRELL, G.W., WARD, B.G., XING, P.-X. & MCKENZIE, I.F.C. (1991). Production of MUC1 and MUC2 mucins by human tumor cell lines. Biochem. Biophys. Res. Commun., 178, 593–599.

DELINE, P.L. & BIRRELL, G.W. (1992). A method for screening large numbers of samples on immunoblots. J. Immunol. Meth., 149, 143–144.

DELINE, P.L. & MCKENZIE, I.F.C. (1992). Mucins: structure, function, and association with malignancy. BioEssays, 14, 619–625.

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PERINI, J.-M., MARIANNE, T., LAFITTE, J.-J., LAMBLIN, G., ROUSSEL, P. & MAZZUCA, M. (1989). Use of antiseraum against glycosylated human mucins for cellular localization of their peptide precursors: antigenic similarities between bronchial and intestinal proteins. J. Histochem. Cytochem., 37, 657.

PODOLSKY, D.K., FOURNIER, D.A. & LYNCH, K.E. (1986). Human colonic goblet cells. Demonstration of distinct subpopulations defined by mucin-specific monoclonal antibodies. J. Clin. Invest., 77, 1263–1271.

PORCHET, N., VAN CONG, N., DUFFOSSE, J., AUDIE, P.J., GUYONNET-DUPERAT, V., GROSS, M.S., DENIS, C., DEGAND, P.L., BERNHEIM, A. & AUBERT, J.P. (1991). Molecular cloning and chromosomal localisation of a novel human tracheo-bronchial mucin cDNA containing tandemly repeated sequences of 48 base pairs. Biochem. Biophys. Res. Commun., 178, 414–422.

PRICE, M.R., PUGH, J.A., HUDEZEC, F., GRIFFITHS, W., JACOB, E., SYMONDS, I.M., CLARKE, A.J. & BLYDWN, R.W. (1990). C95 – a monoclonal antibody against the protein core of human urinary epithelial mucin commonly expressed in breast carcinomas. Br. J. Cancer, 61, 681–686.

PRICE, M.R., SEKOWSKI, M., YANG, G.-L., DURRANT, L.G., ROBINS, R.A. & BLYDWN, R.W. (1991). Reactivity of anti-human gastric carcinoma monoclonal antibody with core-related peptides of the gastrointestinal mucin antigens. J. Biol. Chem., 266, 3998–4002.

GROSS, E. (1967). The cyanogen bromide reaction. Meth. Enzymol., 11, 228–255.

GUM, J.R., BYRD, J.C., HICKS, J.W., TORIBARA, N.W., LAMPORT, D.T.A. & KIM, Y.S. (1989). Molecular cloning of human intestinal mucus cDNAs. Sequence analysis and evidence for genetic polymorphism. J. Biol. Chem., 264, 6480–6487.

HODGES, R.S. & MERRIFIELD, R.B. (1975). Monitoring a solid phase peptide synthesis by an automated spectrophotometric p-nitroanilide method. Anal. Biochem., 65, 241–272.

HOUGHTON, R.A. (1985). General method for the rapid solid phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids. Proc. Natl Acad. Sci. USA, 81, 3998–4002.

HUMAN GENE MAPPING Nomenclature Committee (1989). Tenth International Workshop on Human Gene Mapping. Cytogenet. Cell Genet., 51, 13–66.

JANY, R.H., GALLUP, M.W., YAN, P.S., GUM, J.R., KIM, Y.S. & BASBAUM, C.B. (1991). Human bronchus and intestine express the same mucin gene. J. Clin. Invest., 87, 76–82.

LAYTON, G.T., STANWORTH, D.R. & AMOS, H.E. (1987). The specificity of murine polyclonal and monoclonal antibodies to the haptenic drug chlorozepoxide induced by chlorone-generated chlorophenylalanine protein conjugates. Clin. Exp. Immunol., 69, 157–165.

LAYTON, G.T., DEVINE, P.L., WARNEN, J.A., BIRRELL, G., XING, P.-X., BROWN, B.G. & MCKENZIE, I.F.C. (1990). Monoclonal antibodies reactive with the breast carcinoma-associated mucin core protein repeat sequence peptide also recognise the ovarian carcinoma-associated sebaceous gland antigen. Tumor Biol., 11, 274–286.

MCGUICKIN, M.A., OWENS, M., WRIGHT, R.G., MCKENZIE, I.F.C. & WARD, B.G. (1990). Demonstration of seven-tumor-associated antigens in epithelial ovarian cancer by immunohistochemistry using monoclonal antibodies. J. Tumor Marker Oncol., 5, 87–94.

MCKENZIE, I.F.C. & XING, P.-X. (1990). Mucins and breast cancer: recent immunological advances. Cancer Cells, 2, 75–78.