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Expression in normal adult, fetal, and neoplastic tissues of a carbohydrate differentiation antigen recognised by antigranulocyte mouse monoclonal antibodies

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SUMMARY The distribution in paraffin fixed human tissues of a carbohydrate antigen defined by two monoclonal antibodies raised against human granulocytes has been studied by means of an immunoperoxidase technique. In addition to granulocytes, the antigen has been detected in adult tissues on identifiable cell types of the stomach, kidney, adrenal medulla, and brain and on the mucins of the gastrointestinal tract and other secretions. In fetal tissue, epithelial cells of the alimentary tract, lung, brain, and kidney express the antigen. Adenocarcinoma of the colon, stomach, breast, and lung are stained strongly, as are other types of lung cancer. The monoclonal antibodies give a staining pattern similar but not identical to other monoclonal antibodies raised against granulocytes or neoplastic cell lines which recognise the antigen 3-fucosyl N-acetyllactosamine. The use of antibodies against this oncofetal antigen in the study of differentiation and as tumour markers is discussed.

Monoclonal antibodies are being used increasingly in the study of differentiation and in the diagnosis and differential diagnosis of malignant disease. Ideally, such antibodies would recognise antigens expressed only at one stage in the differentiation of a tissue or in neoplastic but not in normal tissue from the same organ. When mice or rats are immunised with intact cells or membrane fractions, however, antibodies against glycolipids or glycoproteins with a high carbohydrate content are commonly produced and these carbohydrate antigens can be found on a variety of different cell types with no obvious relation, although the expression of the antigen is highly specific within a tissue.[1 2]

A large number of mouse and rat monoclonal antibodies raised independently against a wide range of cell types such as human granulocytes[3 4] or myelomonocytic cell lines,[5 6] several carcinomas of the lung,[7 8] adenocarcinoma of the colon and stomach,[3 9] and a mouse embryonal cell line[10 11] have been shown by radioimmunoassay to recognise the same carbohydrate antigen, 3-fucosyl N-acetyllactosamine. These different monoclonal antibodies have been used independently in the study of embryogenesis, as markers of myeloid differentiation and leukaemia, and as putative markers of neoplasia in stomach, lung, and gut. Although the antibodies all bind to the same oligosaccharide in radioimmunoassays, limited comparison of their specificity by immunohistochemistry has suggested clear differences in the antigenic determinants they recognise. For example, some of the antibodies bind to both granulocytes and monocytes while others are granulocyte specific.[3 6]

No extended comparison of the histochemical specificity of the different monoclonal antibodies has been made. We have therefore investigated the distribution of the antigen recognised by two such antibodies in a wide variety of normal adult, fetal, and neoplastic tissues encompassing all reported binding sites for antibodies recognising 3-fucosyl N-acetyllactosamine. The results allow comparison with those reported for other monoclonal antibodies thought to recognise this antigen.The monoclonal antibodies used in this study were raised against purified human granulocytes. They have similar properties to granulocyte specific antibodies produced in other laboratories[3 12–14] and to monoclonal antibodies available commercially from several sources, which are therefore likely to be used

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increasingly. The striking stability of the antigens to most fixation techniques makes them convenient for use in histopathology.

Material and methods

Monoclonal antibodies MC2 and MC4 were produced from Balb/c mice immunised three times intraperitoneally and intradermally with purified human granulocytes. The production and characterisation of these antibodies has been described previously.[4] The cell lines producing these monoclonal antibodies have been cloned three times and are stable to freezing and thawing and to passage through mice. For this study the ascitic fluid was used as a source of antibody. Control ascitic fluid was produced from the NS 1 cell line, which is the parent myeloma of the MC2 and MC4 hybridoma lines.

A partially purified mouse IgM fraction was prepared by gel filtration of normal mouse serum on Sepharose-6B.

TISSUE SAMPLES

The material used in this study was obtained from the routine histopathology and necropsy services of the Department of Histopathology, Ninewells Hospital, Dundee. All the material described was fixed in Lillie's buffered formalin and embedded in paraffin wax.

IMMUNOPEROXIDASE STAINING

The paraffin embedded sections were taken to water and then endogenous peroxidase activity was blocked by immersion in 0·5% hydrogen peroxide, 0·074% HCl in absolute ethanol for 30 min at room temperature with stirring. The sections were then washed in Tris buffered saline (TBS) for 10 min, and this was followed by three 10 min washes in TBS/1·0% bovine serum albumin/0·1% NaN_3 (TBS/BSA). Monoclonal antibody or control ascites diluted 1/1000 in TBS/BSA was then incubated with the section for 3 h at room temperature and the excess reagent removed by four washes with TBS/BSA. Sheep antimouse IgM-peroxidase conjugate (Serotec) diluted 1/20 in TBS/BSA/10% normal human serum was then added, and after incubation for 30 min the sections were washed three times with TBS/BSA and once with TBS. Peroxidase was detected using a solution of 6·0 mg/ml diaminobenzidine hydrochloride in 0·2 M Tris-HCl, pH 7·6/0·03% hydrogen peroxide, incubated at room temperature for 5 min, and then washed with TBS followed by water. Sections were then counterstained with haematoxylin and blued in Scott's tap water substitute before dehydration and mounting in butylphthalatestyrone.

Unless otherwise stated, two examples of each tissue were examined. Each tissue section was studied using both MC2 and MC4. For control slides, the monoclonal antibody was replaced by an equivalent dilution of NS 1 ascitic fluid to which had been added partially purified mouse serum IgM at 10 times the concentration of IgM in the ascitic fluid containing monoclonal antibody.

Results

Monoclonal antibodies MC2 and MC4 were raised in mice immunised with purified peripheral blood granulocytes. The antibodies have been shown by radioimmunoassay, autoradiography, and

Expression of MC2/4 in normal human tissues

Tissue	*Positive*	*Negative*
Haemopoietic/lymphoid		
Peripheral Blood	Polymorphonuclear leucocytes	Erythrocytes, monocytes, lymphocytes, platelets all other cells
Lymph node,* spleen*		
Alimentary tract	Stomach*	Salivary gland,* oesophagus, liver (hepatocytes and Kupffer cells), gall bladder,* pancreas
	Ileum (mucin only)	
	Colon (mucin only)*	
Urinary tract	Kidney* (cortex)	
Reproductive system	Endometrium (glandular secretion)	Testis, epididymis, vas deferens
Cardiovascular system		Heart, veins, arteries
Respiratory tract		Trachea, bronchi, lung alveolar macrophages
Skin*/muscle	Sweat gland ducts	Epidermis, connective tissue, skeletal muscle
Mammary gland*	Secretion within ducts	
Endocrine glands	Adrenal medulla* (few cells)	Adrenal cortex, thyroid,* pancreatic islets, parathyroid*
Nervous system	Central nervous system—astrocytes in all parts of brain, granular layer of cerebellum	Neurones, ongodendrocytes microglia, ependyma, pineal peripheral nerve

*Tissues have also been studied in frozen sections, giving the same results as fixed embedded tissue.

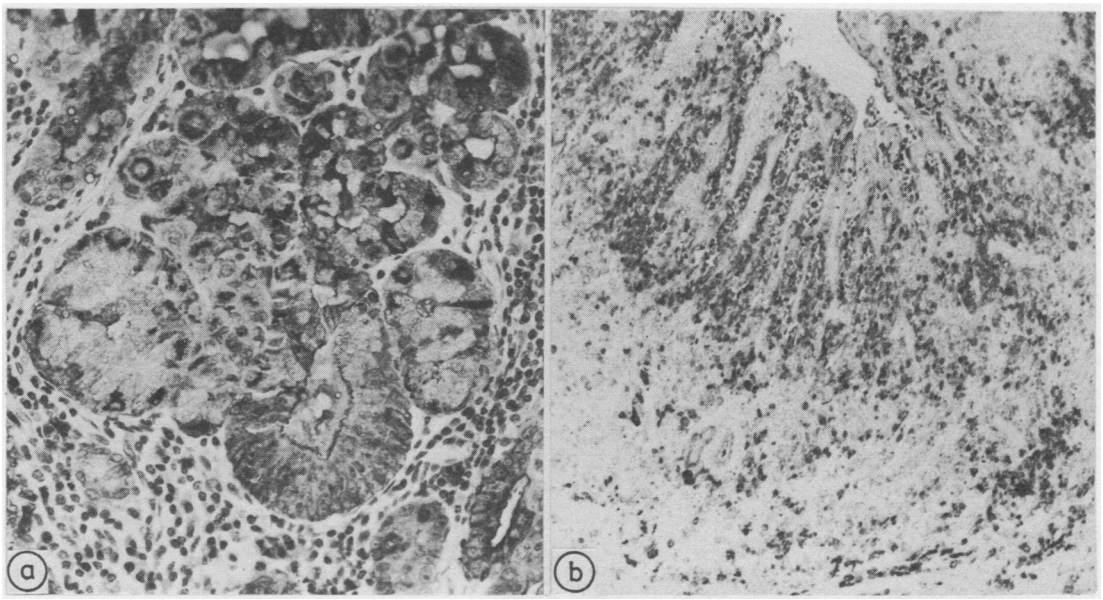

Fig. 1 *(a) Stomach (chronic follicular gastritis). Staining of luminal surface of mucous neck cells, cytoplasm of chief cells, and canaliculi of parietal cells. No staining of associated lymphoid areas. Original magnification × 300. (b) Gastric adenocarcinoma. Staining of the carcinoma throughout the mucosa, submucosa, and muscularis propria. Original magnification × 60. All Figures are indirect immunoperoxidase using monoclonal antibody MC2 and sheep antimouse IgM-peroxidase conjugate. Haematoxylin counterstain.*

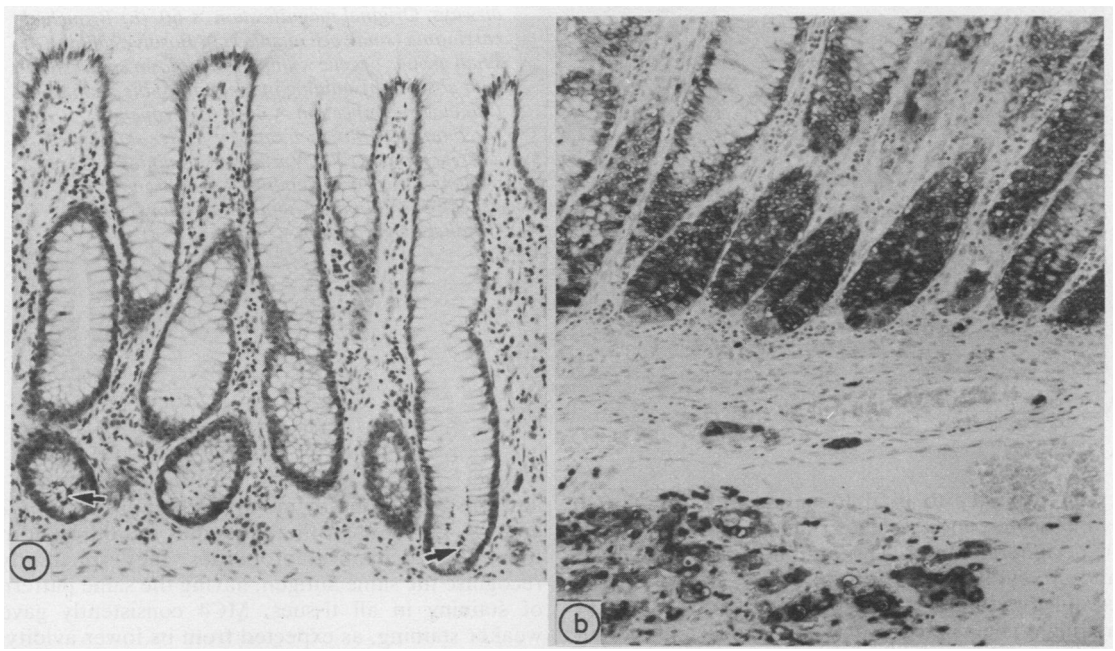

Fig. 2 *(a) Normal colon. Positive staining of surface mucin in gland crypts. Original magnification × 250. (b) Colonic adenocarcinoma. Positive staining of surface mucin and also intracytoplasmic staining of infiltrating component. Note also increased positive staining in overlying (non-neoplastic) colonic gland crypts. Original magnification × 60.*

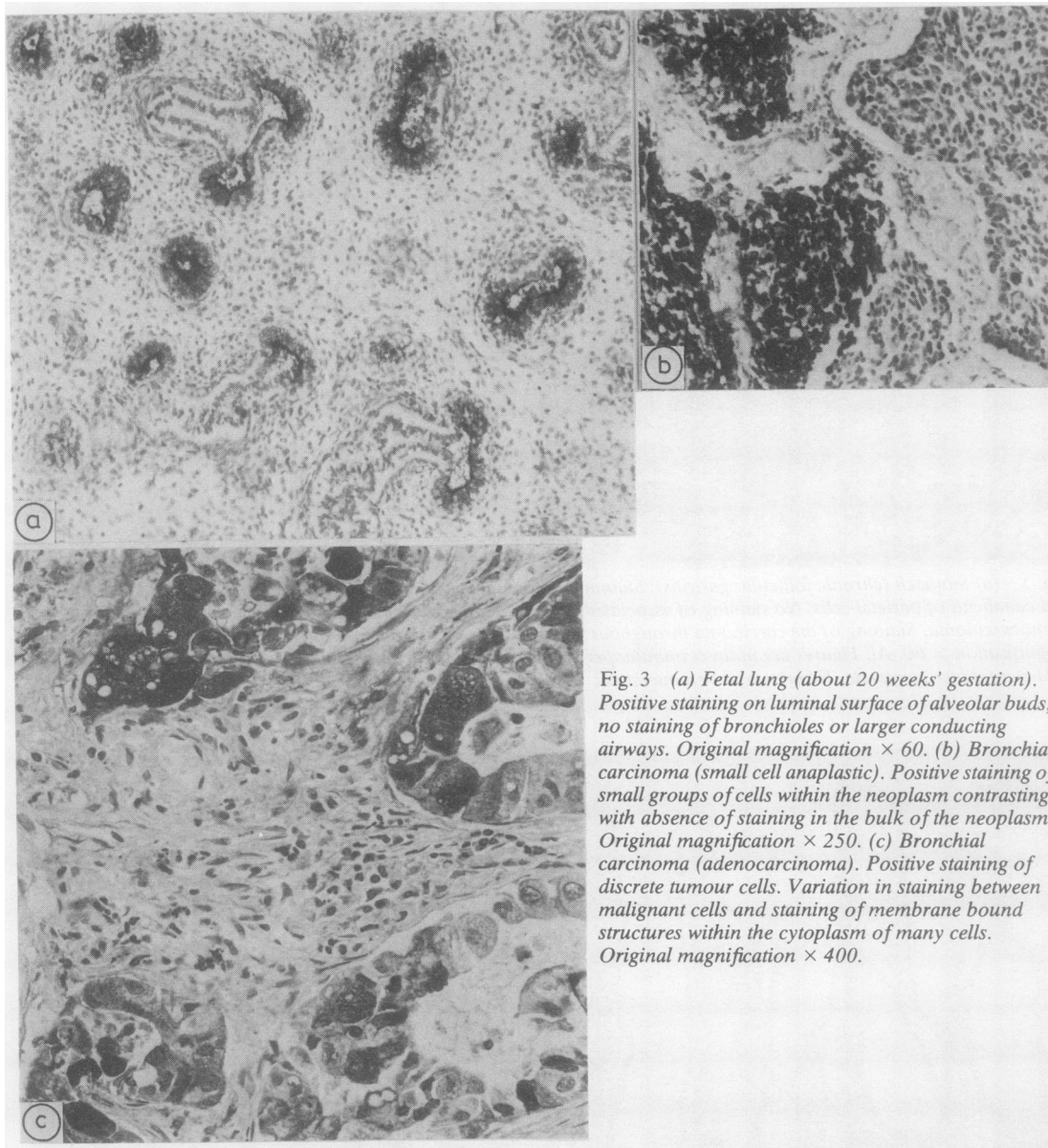

Fig. 3 *(a) Fetal lung (about 20 weeks' gestation). Positive staining on luminal surface of alveolar buds, no staining of bronchioles or larger conducting airways. Original magnification × 60. (b) Bronchial carcinoma (small cell anaplastic). Positive staining of small groups of cells within the neoplasm contrasting with absence of staining in the bulk of the neoplasm. Original magnification × 250. (c) Bronchial carcinoma (adenocarcinoma). Positive staining of discrete tumour cells. Variation in staining between malignant cells and staining of membrane bound structures within the cytoplasm of many cells. Original magnification × 400.*

cytofluorimetry to bind to neutrophils but not to lymphocytes, monocytes, erythrocytes, or platelets in peripheral blood. The antibodies are both IgM and are cytotoxic to neutrophils in the presence of complement but differ in the mobility of their heavy and light chains on polyacrylamide gels and in their avidities, although they compete for the same binding site on the neutrophil cell surface.[4]

The Table summarises the tissue localisation of antigens recognised by monoclonal antibodies MC2 and MC4. Although the antibodies are different in their physicochemical properties, they apparently recognise the same antigen, having the same pattern of staining in all tissues. MC4 consistently gave weaker staining, as expected from its lower avidity. In all of the tissues studied neutrophils were stained strongly. Monocytes, lymphocytes, erythrocytes, or platelets did not express the antigen. Tissue

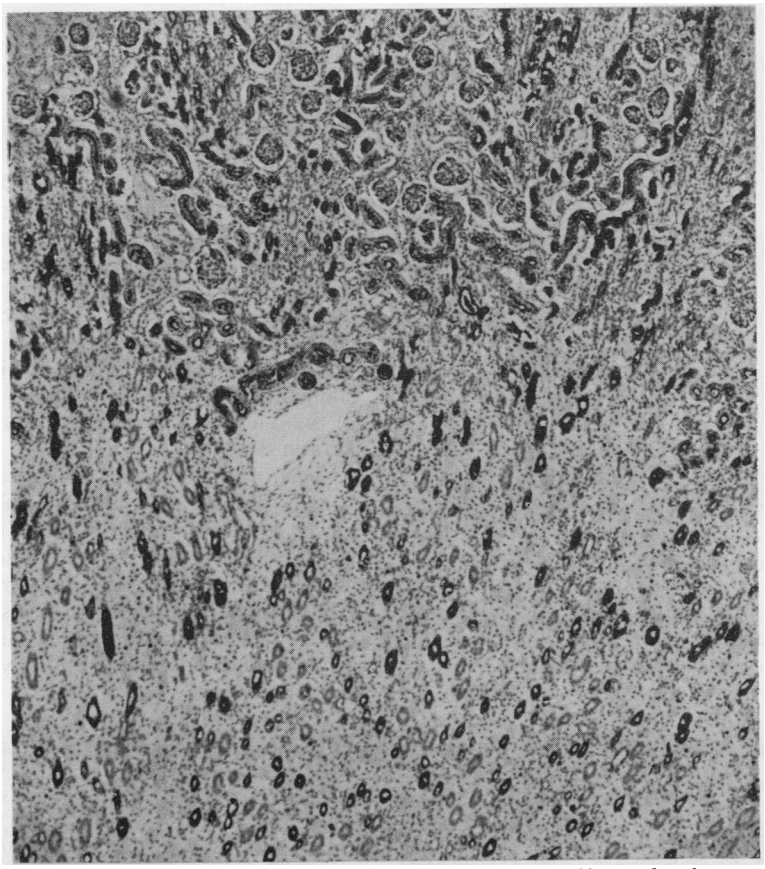

Fig. 4 *Fetal kidney (29 weeks' gestation). Positive staining of luminal and intercellular borders in proximal tubules, luminal staining in Henle's loop. Original magnification* × *60.*

macrophages such as histiocytes, Kupffer cells, alveolar macrophages, and accessory cells in lymph node or spleen were never stained.

In addition to these haemopoietic cells, several other cell types in different tissues were recognised by the antibodies. Most striking was the staining of epithelial cells and secreted mucins. In the stomach, the antigens were detected on surface mucin, the cytoplasm of mucous neck cells, the cytoplasmic granules of chief cells, and parietal cell canaliculi (Fig. 1a). In the early fetus the developing gastric mucosa and meconium were strongly stained. Strong cellular staining was also seen in gastric adenocarcinoma (Fig. 1b).

Mucin was strongly stained in the base of the crypts of both large and small bowel; the epithelial cells themselves were not labelled (Fig. 2a). This again contrasted with definite positive cytoplasmic staining of the cells of colonic adenocarcinoma (3/3). Increased antigen expression was also noted in regions of apparently normal epithelium adjacent to an adenocarcinoma (Fig. 2b). An adenomatous polyp of the large bowel showed only weak surface mucus staining. The antigen was not detected in cells or secretions of the oesophagus or gall bladder. In salivary gland, where the neutrophils in the blood vessels were clearly shown, the antigen was not detected in either acini or ducts. Fetal oesophageal mucosa did express the antigen. No staining was noted in adult liver, in hepatocytes or Kupffer cells, but occasional cells in the hepatic sinusoids of fetal liver were positive. These were thought to be primitive myeloid cells.

The glandular secretion of the secretory endometrium reacted with the monoclonal antibodies, although the epithelial cells and stromal K cells were

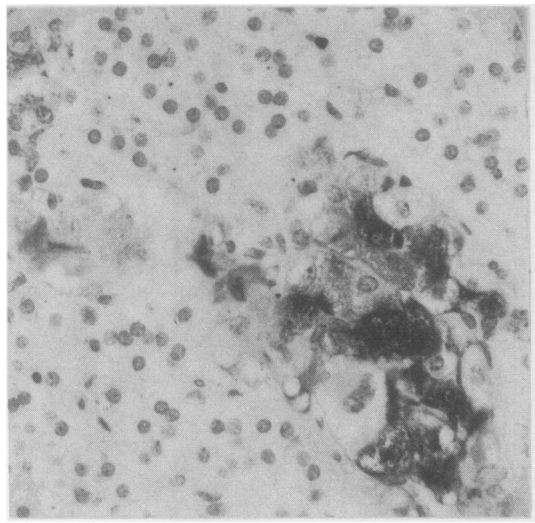

Fig. 5 *Adrenal medulla. Positive granular staining of discrete cells in adrenal medulla. Original magnification* × *300.*

unreactive. No part of the testis, vas deferens, or epidymis was stained. Secretions within the ducts and the luminal surface of epithelial cells of the mammary gland were stained for the antigen. This staining was poor compared with the cellular staining of an invasive ductal carcinoma. But it was apparent that not all the cells of the tumour were stained.

No part of the heart expressed the antigen, nor did the trachea, bronchi, parenchymal cells of the lung, or alveolar macrophages. This was in contrast to the strong staining of alveolar buds in the fetal (4–6 weeks) lung (Fig. 3a). Three types of lung cancer showed staining. An adenocarcinoma was strongly positive, staining large granular organelles within the cytoplasm (Fig. 3b). A small cell carcinoma showed strong staining in some parts, but others, apparently identical in histology, were not stained at all (Fig. 3c). A squamous cell carcinoma of the lung was positive in the tumour nests and in keratin deposits.

Cells of the proximal tubule of the kidney were heavily stained, the antigen being localised on the glycocalyx of the brush borders. Distal tubules and glomeruli were not stained, although parts of the loop of Henle showed positive material. In a fetal kidney the staining pattern was essentially the same except that the transitional epithelium of the renal calyces was stained more strongly than in the adult (Fig. 4).

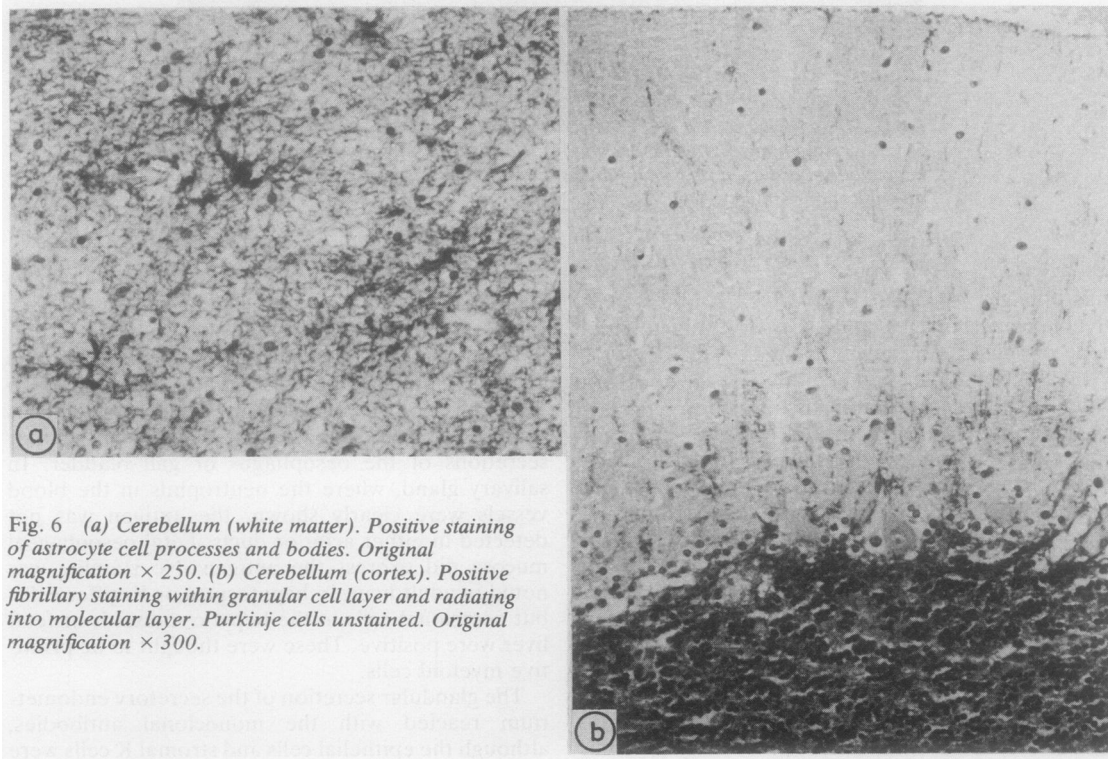

Fig. 6 *(a) Cerebellum (white matter). Positive staining of astrocyte cell processes and bodies. Original magnification* × *250. (b) Cerebellum (cortex). Positive fibrillary staining within granular cell layer and radiating into molecular layer. Purkinje cells unstained. Original magnification* × *300.*

Clear staining of a minor population of cells in the adrenal medulla was noted (Fig. 5). Staining was granular and appeared to be associated with chromaffin granules. No staining of adrenal cortex, thyroid, or pancreas was detected. A neuroblastoma did not express the antigen.

No reactivity was detected in skeletal muscle, cartilage, or in the epidermis, although the secretions of the sweat gland ducts were reactive. Clear staining of many parts of the central nervous system was seen, but the antigen was not expressed in the peripheral nervous system. In brain, astrocytes were reactive in all regions studied (Fig. 6a). The ependyma, choroid plexus, and pineal were not stained. In the cerebellum, in addition to astrocytic staining, the granular layer was positive in the adult brain (Fig. 6b). A medulloblastoma showed no antigen expression.

Discussion

We have shown the widespread expression of the antigen recognised by two antigranulocyte monoclonal antibodies in non-haemopoietic tissues. This result confirms and extends the observations of Schienle *et al*[15] and Howie *et al*,[16] who carried out histochemical studies using different monoclonal antibodies. The main reactivity to each of the monoclonal antibodies was detected in epithelia, the kidney, and in the central nervous system. There are, however, some clear differences in the specificities of the antibodies—for example, MC2 and MC4 did not stain the epithelium of oesophagus, acini of pancreas, or salivary gland, the ducts of salivary gland, or hepatocytes. These differences are unlikely to reflect differences in technique; it is more likely that the monoclonal antibodies recognise different antigenic determinants. The possibility that a series of closely related antigenic determinants is recognised by different monoclonal antibodies is borne out by Howie *et al*, who showed that the monoclonal antibody AG4·36, which recognises immature granulocytes in bone marrow but not mature neutrophils, gave a similar staining pattern in all tissues tested to the antibody AG4·48, which recognises only mature neutrophils. It is interesting that these monoclonal antibodies should show such narrow specificity when binding to leucocytes but still all recognise the same wide range of cell types in other tissues.

We have now extended the observations on the tissue distribution of granulocyte antigenic determinants to a range of fetal and neoplastic tissues. In the fetus most tissues showing staining of mucin or cells in adults showed pronounced cellular staining in the developing mucosa. A notable exception was the lung, where the expression of antigen in the fetal alveolar buds was lost completely on maturity. In an extended study, which will be reported more fully elsewhere, we have shown that in fetal and neonatal cerebellum, expression of the antigen has clear developmental regulation. At 30 weeks' gestation there was little staining of any of the three cortical layers (IGL, EGL, ML). At 35 weeks' gestation there was weak positive staining of fibrillary material within the molecular layer. At 40 weeks' gestation this staining had increased and there was fibrillary staining within the internal granular layer, similar to that seen in the adult.

The antigens detected by MC2 and MC4 are expressed in large amounts of adenocarcinoma of colon, stomach, lung, and breast, tissues where no staining or staining due to mucin secretion is seen in the normal tissues. These antigens can therefore be included in the increasing list of carbohydrate oncofetal antigens. The tissue distribution of the antigens in normal and neoplastic tissue shows a striking similarity to the oncofetal antigen (SSEA-1), which is recognised by a monoclonal antibody raised against a mouse teratocarcinoma cell line.[10] Again, however, clear differences are observed, chiefly in the ability of the anti-SSEA-1 to recognise all members of the monocyte/macrophage cell lineage[17 18] and its reactivity with salivary gland. Monoclonal antibodies against SSEA-1 were the first to be shown by radioimmunoassay to recognise the carbohydrate structure 3-fucosyl N-acetyllactosamine.[11 19] The same oligosaccharide is also recognised by MC2 and MC4 (T Feizi, personal communication) and other granulocyte or granulocyte/monocyte specific antibodies. Radioimmunoassays are restricted by the limited availability of purified oligosaccharides and histochemical analysis is clearly able to detect subtle changes in the way the antigen is expressed. The carbohydrate antigen recognised by SSEA-1, MC2, and MC4 and several other monoclonal antibodies is carried on granulocytes by glycoproteins of molecular weight 150 000 and 105 000 (Albrechtsen M, Kerr MA, unpublished observations).[3 20]

3-fucosyl N-acetyllactosamine was first identified as part of lactofucopentaose III, a carbohydrate found in human milk[21] and subsequently in adenocarcinoma.[22] Many monoclonal antibodies against adenocarcinoma of the colon or stomach, several types of lung cancer, or against teratocarcinoma cell lines also recognise this antigen. Indeed, it has been suggested that this oligosaccharide is the immunodominant antigen on many tumours and therefore the antigen recognised by many "tumour specific" monoclonal antibodies.[8 9] Since the antigen is stable to many fixation techniques and is present in large amounts on many tumours, while being pre-

sent in much smaller amounts and in a localised manner on the normal tissue, these monoclonal antibodies are attractive as histochemical tumour markers. In addition, we and others have shown that apparently normal tissue adjacent to tumour can also be strongly positive for the antigen, which suggests the identification of a premalignant state. It must be emphasised, however, that both our work and that of Solter and Knowles have shown that within a carcinoma both positive and negative cells occur and that not all adenocarcinomas are positive. Similar caution must also be applied to other tumour markers such as carcinoembryonic antigen,[24] epithelial membrane antigen,[25] and the monoclonal antibody Ca19·9, which recognises a closely related fucosylated oligosaccharide.[26 27] The possible use of monoclonal antibodies recognising 3-fucosyl N-acetyllactosamine in the diagnosis of tumours or identification of secondaries suggests the need for a more extensive study.

Although the antigen recognised by MC2 and MC4 shows a widespread expression in human tissues, it is clear that within any tissue expression is restricted. The antibodies therefore recognise useful differentiation antigens. In blood, where only granulocytes are reactive, these monoclonal antibodies can be used routinely for cell and leukaemia typing. In the adrenal medulla, pituitary, and stomach, secretory granules of specific cells are labelled. In brain the antigens appear to be surface antigens of astrocytes. The expression of the antigen is also clearly developmentally regulated in lung and in the cerebellum. It would therefore seem most likely that these antibodies will be of considerable use in the study of differentiation of these tissues. Although surface carbohydrate has often been suggested to be important in cell to cell contact, the actual mechanism of action of these structures on the cell surface is almost unknown. The availability of monoclonal antibodies against this relatively well defined carbohydrate structure will be important in studies on the role of carbohydrate in cell communication and in the changes which occur during transformation.

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