action was the pluripotential haemopoietic stem cell (CFU-S). Whenever they were tested, free glycosaminoglycan chains were unable to reproduce β-D-xylolides influences on cell behaviour. On the basis of the cited biochemical evidence it seems likely that the xylolides effects are mediated through alterations in surface membrane proteoglycans. Most probably the xylolide-induced changes follow from a reduction in proteoglycan concentration in the extracellular matrix and/or the associated tissue fluids. It should also be remembered that, in contrast to the humble sea urchin, xylolides influence on the formation of nuclear proteoglycans in mammalian systems have not been investigated and represent an important area for future studies. Nevertheless, the use of β-D-xylolides has emphasized the great significance of proteoglycans in normal cellular proliferation and morphogenesis and these sugar derivatives will remain powerful tools for further experiments at the molecular and cellular levels.


Carbohydrate differentiation and cancer-associated antigens detected by monoclonal antibodies

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During differentiation and oncogenic transformation the structures of complex carbohydrates on the cell surface change as result of a modulation of the levels of glycosyltransferases. Many monoclonal antibodies which detect differentiation or transformation antigens are directed against these carbohydrates. Of 325 monoclonal antibodies that we have obtained from different laboratories, 97 bind carbohydrates (Table 1).

Cell-surface carbohydrates can exist in either glyco-proteins or glycolipids and in many cases identical oligosaccharides are found on both types of molecules. As glycolipids have a relatively simple structure and contain one hapten oligosaccharide, they are ideal for the chemical characterization of cell-surface-carbohydrate antigens.

A gastrointestinal cancer-associated antigen detected by monoclonal antibody 19-9 is a glycopeptide containing sialylated lacto-N-fucopentaose II in colorectal carcinoma cell line SW 1116

Monoclonal antibody 19-9, produced by hybridomas obtained from mice immunized with a human colon adenocarcinoma cell line, has an apparent specificity for human intestinal tumours (Koprowski et al., 1979). Binding of this antibody to tumour cells is inhibited by serum from most patients with pancreatic and gastrointestinal cancer, but not by the serum of normal individuals, patients with inflammatory bowel diseases, or most patients with other malignancies (Herlyn et al., 1982). The antigen for this antibody in the cell line used for immunization is a monosialoganglioside (Magnani et al., 1982) and was purified with the aid of a method we developed that allows the visualization of glycolipid antigens by direct binding of radioactive antibodies to thin-layer chromatograms followed by autoradiography (Magnani et al., 1981, 1982). About 30 µg of ganglioside antigen is obtained from 1 g wet weight of tissue culture cells.

The structure of the carbohydrate was determined by methylation analysis, and combined gas chromatography and mass spectrometry of the triluroacetylated derivative (Magnani et al., 1982), to be:

\[
\text{NeuNac}\text{2}-3\text{Galf}-3\text{GlicNAcBf-4Glic}\n\]

\[\text{Fuc}z\]

This is a sialylated derivative of the normal Le" blood group-active pentasaccharide, lacto-N-fucopentaose II. Both fucose and sialic acid are immunodominant sugars as neither lacto-N-fucopentaose II nor LS-tetrasaccharide a (the defucosylated sialyloligosaccharide) bind the antibody.
Table 1. Monoclonal antibodies directed against carbohydrates

<table>
<thead>
<tr>
<th>Number of antibodies</th>
<th>Antigen</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>(out of 325 tested)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>H type 1</td>
<td>Fucα1-2Galβ1-3Glc (\text{N}Ac) . . .</td>
</tr>
<tr>
<td>1</td>
<td>H type 2</td>
<td>Fucα1-2Galβ1-4Glc (\text{N}Ac) . . .</td>
</tr>
<tr>
<td>1</td>
<td>Le(\text{a})</td>
<td>Galβ1-3Glc (\text{N}Ac) . . .</td>
</tr>
<tr>
<td>4</td>
<td>Le(\text{b})</td>
<td>Fucα1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fucα1-2Galβ1-3Glc (\text{N}Ac) . . .</td>
</tr>
<tr>
<td>3</td>
<td>B</td>
<td>Fucα1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fucα1-2Galβ1-4Glc (\text{N}Ac) . . .</td>
</tr>
<tr>
<td>55</td>
<td>Lacto-N-fucopentaose III</td>
<td>Galβ1-4Glc (\text{N}Ac) . . .</td>
</tr>
<tr>
<td></td>
<td>(also called SSEA-1, My-1, Le(\text{a}) and X-hapten)</td>
<td>Fucα2</td>
</tr>
<tr>
<td>55</td>
<td>Sialylated Le(\text{a})</td>
<td>NeuNαcα2-3Galβ1-3Glc (\text{N}Ac) . . .</td>
</tr>
<tr>
<td>25</td>
<td>Unidentified carbohydrate sequences in glycolipids and/or glycoproteins</td>
<td>Fucα1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fucα1</td>
</tr>
</tbody>
</table>

Total = 97

Sialylated lacto-N-fucopentaose II is present on a mucin in patient’s sera and in normal saliva

Although the sialylated Le\(\text{a}\) sequence of sugars occurs in the gangliosides of pancreatic and gastrointestinal cancers, most of the antigen detected in the sera of patients are mucins (high molecular weight, carbohydrate-rich glycoproteins). The evidence (Magnani et al., 1983) for this is as follows. Little antigen is extracted by organic solvents from sera and that which remains at the origin under conditions of thin-layer chromatography where the ganglioside antigen migrates up the plate. Upon gel filtration of serum on Sephacryl S-400, the antigen elutes in the void volume, indicating a Mr \(\geq 5 \times 10^6\). Incubation for 5 h at 35°C in 0.1M NaOH destroys the antigen but does not affect the ganglioside antigen. The density of the serum antigen as determined in a CsCl gradient is 1.50 g/ml, while in serum antigen in mucins from individuals belonging to the Le\(\text{a}\) blood group its density is 1.43 g/ml. Finally, antigen affinity-purified by anti-sialylated Le\(\text{a}\) antibody from the serum of a cancer patient belonging to the Le\(\text{a}\) blood group contains Le\(\text{b}\) antigen, consistent with the multiple antigenic specificities exhibited by mucins. The occurrence of mucins in the blood of cancer patients has been reported many times (Race & Sanger, 1975).

In a previous study (Magnani et al., 1982), sialylated Le\(\text{a}\) antigen was not detected by solid-phase radioimmunoassay in extracts from normal adult tissues. By immunoperoxidase labelling of normal tissue sections, however, the antigen was found in a layer of ductal cells in normal pancreas and a layer of cells in normal salivary glands and bronchial epithelium that secrete mucins (Atkinson et al., 1982), and by autoradiography low levels of ganglioside antigen were detected in extracts of normal pancreas (Hansson et al., 1983). The antigen is also found in salivary mucins from most normal individuals belonging to the Le\(\text{a}\) blood group and is not found in salivary mucins from normal individuals belonging to the Le\(\text{a}\) blood group (M. Brockhaus, M. Wyorka, J. L. Magnini, Z. Steplewski, H. Koprowski & V. Ginsburg, unpublished work). About 7% of the population belong to the Le\(\text{a}\) blood group because they lack the fucosyltransferase that catalyses the synthesis of the sugar sequence Fucα1-4Glc \(\text{N}Ac\) . . . (Grollman et al., 1969). As a consequence, cancer patients belonging to the Le\(\text{a}\) blood group cannot synthesise sialylated Le\(\text{a}\) antigen (Koprowski et al., 1982). There is more antigen in mucins from individuals belonging to the Le\(\text{a}\) blood group than in mucins from individuals belonging to the Le\(\text{a}\) blood group. This is probably due to a competition between the glycosyltransferase that produces the Le\(\text{b}\) structure and that which produces sialylated Le\(\text{a}\) structure for the same acceptor oligosaccharide. Thus data on the presence of sialylated Le\(\text{a}\) oligosaccharide in sera of cancer patients detected by monoclonal antibody 19-9 should be categorized with respect to Lewis blood group status.

Many monoclonal antibodies which detect differentiation antigens and apparent tumour-associated antigens are directed against a carbohydrate sequence found in lacto-N-fucopentaose III

Another carbohydrate antigen which occurs on both glycoproteins and glycolipids is remarkably immunogenic in mice and rats. As shown in Table 1, about one sixth of the antibodies tested are directed against a sugar sequence found in the human milk oligosaccharide lacto-N-fucopentaose II (Kobata & Ginsburg, 1969). A glycolipid containing lacto-N-fucopentaose III was first isolated from a human adenocarcinoma (Yang & Hakomori, 1971). The same sugar sequence minus the glycosyl residue occurs in higher glycolipids (Hakomori et al., 1982) and also in glycoproteins (Lloyd et al., 1968). Antibodies against this sugar sequence detect a stage-specific embryonal antigen (called SSEA-1) of the murine embryo and teratocarcinoma (Gooi et al., 1981; Hakomori et al., 1982); they also detect an antigen (called My-1) that is strongly expressed in human granulocytes and granulocyte precursor cells but not in normal peripheral blood lymphocytes, monocytes, platelets and erythrocytes (Huang et al., 1983), and they also detect an antigen characteristic of human small cell carcinoma.
adenocarcinoma and squamous cell carcinoma of the lung (Huang et al., 1983).

Recently, many laboratories have described this oligosaccharide antigen as a differentiation marker restricted to the myeloid cells (Andrews et al., 1983; Girardet et al., 1983; Gooi et al., 1983; Huang et al., 1983; J. L. Magnani, E. D. Ball, M. W. Fanger, L. C. Huang, S. Hakomori & V. Ginsburg, unpublished work). In particular, three antibodies (PMN6, PMN29 and PM81) show differences in cell binding, but all recognize an oligosaccharide sequence in lacto-N-fucopentaose III. PMN6 and PMN29 bind specifically to granulocytes but differ in the ability to bind certain cell lines (Ball et al., 1983), while PM81 binds granulocytes, eosinophils, monocytes and most acute myelocytic leukaemia cells (J. L. Magnani, E. D. Ball, M. W. Fanger, L. C. Huang, S. Hakomori & V. Ginsburg, unpublished work). Despite these differences the binding of all three monoclonal antibodies to cells is inhibited by the oligosaccharide, lacto-N-fucopentaose III. Radioimmunoassays using purified glycolipids containing the sugar sequence in lacto-N-fucopentaose III demonstrate different binding characteristics for each monoclonal antibody. PM81 binds lower concentrations of glycolipids than PMN29, while PMN6 requires the highest concentration of glycolipids for binding. Autoradiography of thin-layer chromatographs of glycolipid antigens stained with radioactive antibodies supports these results. The data suggest that the binding of these monoclonal antibodies to cells is dependent on the quantity of lacto-N-fucopentaose III-containing molecules on the cell surface (J. L. Magnani, E. D. Ball, M. W. Fanger, L. C. Huang, S. Hakomori & V. Ginsburg, unpublished work). Each antibody requires a different concentration of antigen for binding. Under this threshold concentration a cell may contain antigen, but will not bind antibodies.


Monoclonal antibodies reveal saccharide structures of glycoproteins and glycolipids as differentiation and tumour-associated antigens

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The last several years have witnessed a resurgence of interest in the identification of antigenic markers which distinguish tumour cells from their normal counterparts, foetal cells from those of adults and one differentiated cell from another. This renewed interest is due to the introduction of the hybridoma technique (Köhler & Milstein, 1975), which has made it possible to produce monoclonal antibodies of desired specificities and to identify with precision the antigens that they recognize. It has been hoped that such ‘tailor-made’ monoclonal antibodies would single out antigenic markers which would be useful for the diagnosis of cancer, the immunotherapy of tumours and the typing of leukaemic cells and not least for detecting important components which change during successive stages of development and differentiation.

Abbreviation used: IgM, immunoglobulin M.

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In this report, I shall briefly review our studies which have shown that a number of such cellular markers recognized by monoclonal antibodies are carbohydrate structures. The antigenic markers in question are as diverse as (a) developmentally regulated antigens of human erythrocytes (Marsh, 1961), (b) an antigenic marker of gastric adenocarcinoma in persons who are secretors of the blood group antigens (Picard et al., 1978; Feizi, 1979; Kapadia et al., 1981a), (c) a marker of human colon adenocarcinoma (Brown et al., 1983), (d) an antigenic marker of human foetal endothelial cells (Williams et al., 1982), (e) distinctive markers of human granulocytes amongst cells of the peripheral blood (Rumpold et al., 1982; Brown et al., 1983), (f) differentiation antigens of human breast epithelium (Foster et al., 1982), (g) stage-specific embryonic antigen of mouse (Solter & Knowles, 1978) and (h) a marker of the primary endoderm of the mouse (Kapadia et al., 1981b). All these antigenic markers are carbohydrate structures carried on various glycoproteins and glycolipids of the cell surface (Feizi, 1981a, 1982a; Table 1). They belong to a family of carbohydrate structures which also includes the major blood group antigens A, B, H, Le and Le" (see Figs 2 and 3).