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; Giradet 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Gooi et al., 1983; Huang et al., 1983; J. L. Magnani, E. D. Ball, M. W. Fanger, L. C. Huang, S. Hakomori & V. Ginsburg, unpublished work).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most acute myelocytic leukaemia cells (J. L. Magnani, E. D. Ball, M. W. Fanger, L. C. Huang, S. Hakomori & V. Ginsburg, unpublished work).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molecules on the cell surface (J. L. Magnani, E. D. Ball, M. W. Fanger, L. C. Huang, S. Hakomori & V. Ginsburg, unpublished work).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

TEN FEIZI
Applied Immunochemistry Research Group, Clinical Research Centre, Watford Road, Harrow, Middx. HA1 3UJ, U.K.

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.

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 endoderm (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, Lea and Leb, (see Figs. 2 and 3).
Table 1. Structures behaving as differentiation or tumour-associated markers in man and mouse

*The i, I and I(Ma) antigens are recognized by natural monoclonal autoantibodies; the other antigens are recognized by hybridoma-derived antibodies. References: (a) Feizi, 1981b; (b) Hakomori, 1981b; (c) Kapadia et al., 1981a; (d) Feizi et al., 1982; (e) Feizi 1982b; (f) Picard et al., 1978; (g) Gooi et al., 1983b; (h) Feizi, 1983a; (i) Gooi et al., 1983a; (j) Gooi et al., 1981; (k) Hounsell et al., 1981; (l) Brown et al., 1983; (m) Gooi et al., 1983c.

<table>
<thead>
<tr>
<th>Antigens</th>
<th>Designation*</th>
<th>Structure</th>
<th>Markers in mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distinctive antigen of foetal erythrocytes</td>
<td>i</td>
<td>Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc</td>
<td>Marker of embryonic endoderm (c, d)</td>
</tr>
<tr>
<td>Distinctive antigen of erythrocytes of adults</td>
<td>I</td>
<td>Galβ1-4GlcNAcβ1 Galβ1-4GlcNAcβ1</td>
<td>Marker of earliest embry cells (e)</td>
</tr>
<tr>
<td>Distinctive antigen of normal stomach mucosa in 'non-secretors' (e, f)</td>
<td>I(Ma), M39, M18</td>
<td>Galβ1-4GlcNAcβ1 Galβ1-4GlcNAcβ1</td>
<td>Marker of eight-cell stage embryo (j, k)</td>
</tr>
<tr>
<td>Tumour-associated antigen in stomach of 'secretors' (e, f)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Differentiation antigen of normal breast epithelium (g)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Distinctive marker of granulocytes among cells of the peripheral blood (h, i)</td>
<td>SSEA-1, VEP8, VEP9</td>
<td>Galβ1-4GlcNAcβ1 Galβ1-4GlcNAcβ1</td>
<td></td>
</tr>
<tr>
<td>Marker of carcinoma of colon</td>
<td>C14</td>
<td>Galβ1-4GlcNAcβ1 Galβ1-4GlcNAcβ1</td>
<td></td>
</tr>
<tr>
<td>Intracellular antigen of granulocytes (l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marker of embryonic endoderm (m)</td>
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**The three carbohydrate domains on blood group-related oligosaccharides**

The blood group-related carbohydrate chains consist of three domains (Fig. 1): 'core', 'backbone', and 'peripheral' (reviewed by Hounsell & Feizi, 1982). Monosaccharides in the core region vary according to whether the oligosaccharides are joined by N- or O-glycosidic linkage to protein (Kornfeld & Kornfeld, 1980; Hounsell & Feizi, 1982) or to lipid (Hakomori, 1981a). The antigens I shall discuss in this report are found associated with the backbones and the peripheral regions. The backbone regions consist of alternating galactose and N-acetylgalactosamine residues joined by two types of linkage with differing antigenicities, known as Type 1 and Type 2 chains (Watkins, 1980) as follows:

- Galβ1-3GlcNAc: Type 1
- Galβ1-4GlcNAc: Type 2

In linear sequence the repeating disaccharide units are joined to one another by 1-3 linkage (Table 1, Figs. 2 and 3); branch points are formed by disaccharide units joined either by 1-6 linkage to galactose or to N-acetylgalactosamine residues in the backbone or core regions respectively. Backbone regions are extremely variable (Hounsell & Feizi, 1982) and may be longer or shorter than shown in Fig. 1 and they may be linear or branched in different cell types; their antigenicity varies accordingly. The backbone sequences may be further glycosylated with monosaccharides such as fucose, galactose or N-acetylgalactosamine to give rise to peripheral regions with differing antigenicities. Among these the best known are the major blood group antigens, A, B, H, Lea and Leb (Watkins, 1980). The peripheral glycosylations often mask the antigenicities of the underlying backbone structures (Feizi, 1981a, b).

It is of considerable interest that there occur both natural and hybridoma-derived antibodies against this family of saccharide structures. Certain of these structures behave as tumour-associated antigens in some individuals but not in others (Picard & Feizi, 1983). Furthermore, certain of these antigens are normal cellular components of some tissues but they behave as tumour-associated antigens in others (Feizi, 1983c). The unexpected aspect of all these findings (and this is being supported by observations from other groups) is that no unique structures have been revealed thus far.

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**Tumour-associated and differentiation antigens associated with Type 2 backbone sequences**

In the sera of patients with the autoimmune haemolytic disorder known as cold agglutinin disease, there occur high titre monoclonal autoantibodies known as anti-I and anti-cold agglutinins. It so happens that the I and i antigens recognized by these natural monoclonal antibodies are developmentally regulated carbohydrate structures (Feizi, 1981b; Hakomori, 1981b). The I antigen which is a linear oligosaccharide consists of repeating Type 2 sequences and it is a prominent antigen of human foetal erythrocytes. In contrast it is the branched structure, I antigen, which predominates in the erythrocytes of adults (Table 1).

The I and i sequences serve as backbone structures for the H, A and B antigens (Fig. 2) (Feizi, 1981b; Hakomori, 1981b) which occur not only on erythrocytes but also in various tissues of the body where their expression is developmentally regulated (Szulman, 1980). Of special interest is the mode of expression of the antigens A, B and H and I in the gastric mucosa and the mucosal glycoproteins (gastric mucus) (reviewed by Feizi 1982b; Hounsell & Feizi, 1982). In individuals who are secretors of the major blood group antigens (approx. 75% of the population), the gastric mucosa and gastric mucus strongly express the H, A or B antigens found on their erythrocytes. In contrast, in non-secretors whose epithelial cells lack blood group H-conferred fucosyltransferase (Watkins, 1981), these antigens are lacking in the superficial gastric mucosa and the mucus they secrete. Instead, the I antigen is strongly expressed (Picard et al., 1978; Picard & Feizi, 1983); in particular, the I antigenic determinant consisting of the branch point sequence:

\[
\text{Galβ1-4GlcNAcβ1
\]

recognized by anti-I Ma (Table 1, Fig. 2) is strongly expressed. On the other hand when secretors develop gastric cancer, the I(Ma) determinant is strongly expressed in their tumours (Picard et al., 1978; Picard & Feizi, 1983); this may be due to incomplete biosynthesis of the H, A or B determinants. Thus the I(Ma) determinant can be regarded as a ‘distinctive’ marker of the gastric mucosa of non-secretors but a tumour-associated antigen in the gastric cancer tissues of secretors (Table 1). We are currently measuring the level of the I(Ma) antigen in the gastric juice of secretors in order to evaluate its potential as a diagnostic marker of gastric cancer.

The I, A, and B antigens can be regarded as stage-specific antigens (Kapadia et al., 1981a; Feizi et al., 1982) for the I but not the i antigen is expressed on the earliest mouse embryo, and the i antigen appears precisely at the onset of differentiation when the primary endoderm is formed (Table 1). Thus the antigenic change from i to I during development, as seen with human erythrocytes, is not a general phenomenon and the reverse order may occur.
Fucosylated Type 2 chains may behave as differentiation and tumour-associated antigens

The Type 2 chain when α1-3 fucosylated as in (3-fucosyl-N-acetylactosamine Table 1) behaves as a differentiation antigen in two systems (Table 1, Fig. 2). In the mouse this determinant (Gooi et al., 1981; Hounsell et al., 1981) is the eight-cell stage-specific embryonic antigen, known as SSEA-1, recognized by a monoclonal antibody raised by Solter & Knowles (1976) against the F9 teratocarcinoma cell line of the mouse. However, in man the same trisaccharide structure behaves (Feizi, 1983a; Gooi et al., 1983a) as a distinctive marker of granulocytes amongst cells of the peripheral blood, as recognized by two monoclonal antibodies, VEP8 and VEP9, raised by Rumpold et al. (1982). It appears that approx. 50% of monoclonal antibodies against human myeloid cells recognize this determinant (H. C. Gooi, W. Knapp & T. Feizi, unpublished work); these include the monoclonal antibody VIM-D5 and several other monoclonal antibodies, VIM-4, VIM-7, VIM-9 VIM-C6 and VIM-6, raised by Knapp et al. (1983). The remaining 50% of the myeloid cell-specific monoclonal antibodies including VIM-2 (Knapp et al., 1983) recognize saccharide structures which are distinct from 3-fucosyl-N-acetylactosamine (K. Uemura, B. Macher, W. Knapp & T. Feizi, unpublished work). A number of other laboratories have also reported monoclonal antibodies against myeloid cells which have specificity for 3-fucosyl-N-acetylactosamine, as discussed by Magnani et al. (1984). Mice immunized with a number of human tumours have also produced antibodies with specificity for this structure (Hansson et al., 1983). It is of interest that this antigen is not expressed as a granulocyte antigen in the mouse (S. Thorpe & T. Feizi, unpublished work). Thus, apart from individual and tissue differences in the expression of the carbohydrate differentiation antigens (Picard & Feizi, 1983), there are marked species differences.

At later stages of development the Ii and SSEA-1 antigens can no longer be detected in the majority of the tissues of the mouse (Kapadia et al., 1981a; Feizi et al., 1982). In certain epithelial tissues the blood group H antigen is strongly expressed (Kapadia, 1981a). Since the fucose residues associated with blood group H antigen mask the antigenicity of the Ii and SSEA-1 structures (Feizi et al., 1971; Gooi et al., 1981; Hounsell et al., 1981), we envisage that the apparent disappearance of the latter antigens may be due to their being masked by additional fucosylation or other substitutions. On the other hand the resulting difucosyl Type 2 chains (Table 1, Fig. 2) express a tumour-associated antigen of human colon carcinom epithelium as recognized by the hybridoma antibody C14 (Brown et al., 1983). This antigen is, in addition, expressed as an intracellular antigen of human granulocytes (Brown et al., 1983; A. Brown, S. Thorpe & T. Feizi, unpublished work).

The interrelationships of these several antigens including the Type 2 based blood group H, A and B antigens are illustrated in Fig. 2.

The Type 1 backbone structure as a marker of undifferentiated teratocarcinomas and foetal endodermal tissues of man

Undifferentiated teratocarcinomas and endodermal tissues of the human foetus express an antigen recognized by a hybridoma antibody FC 10.2 which was produced by Williams et al. (1982), following immunization of mice with formalin-fixed human embryonal carcinoma line LICR LON HT 39/7. We have shown (Gooi et al., 1983c) that the antigenic determinant recognized by this antibody involves the N-acetylactosamine, Table 1. This antibody shows little reaction with endodermal tissues of the adult, although the Type 1 sequence is well known to occur on glycoproteins (Hounsell & Feizi, 1982) and glycolipids (Karlsson & Larson, 1981) of the gastrointestinal tract. This is because the Type 1 sequence becomes α1-4 fucosylated and converted to the Lea antigen or α1-2 fucosylated and converted to the blood group H antigen or alternatively it may be difucosylated and converted into the Lea antigen as shown in Fig. 3 (Watkins, 1980). These various glycosylations result in the masking of the FC 10.2 determinant. Presumably in the human foetus and in the undifferentiated teratocarcinoma cells a high proportion of the backbones are unsubstituted. The carbohydrate specificity of FC 10.2 antibody resembles that of a human Waldenström macroglobulin, IgMwoO (Kabat et al., 1982).

For completeness, it should be pointed out that the colon tumour-associated antigen recognized by the monoclonal antibody 19-9 produced in the laboratory of Kropowski has been shown by Magnani et al. (1982) to involve the α2-3 sialylated form of the Lea antigen shown below:

\[
\text{NeuAc}2\text{-3Galβ1-3GlcNAc}1_4\text{Fucα}
\]

Like the other tumour-associated antigens the 19-9 determinant is not confined to colonic adenocarcinomas but it has been reported to occur as a normal component of the pancreas (Hansson et al., 1983).

Glycoprotein and glycolipid carriers of the tumour-associated and differentiation antigens

These blood group-related carbohydrate structures are well known to be carried on glycoproteins and glycolipids. We have recently compared their expression on glycoproteins and glycolipids of erythrocytes, granulocytes, the promyelocytic cell line HL 60, and mouse teratocarcinoma cells (Feizi, 1983; Childs et al., 1983; T. Feizi, unpublished work). Immunostaining of (a) total cell lysates after polyacrylamide-gel electrophoresis and transfer on to nitrocellulose ('Western' blotting) and (b) glycolipid extracts on thin-layer chromatography plates, has revealed that the proportion of glycoproteins and glycolipids carrying these antigens varies in different cell types. Precise quantifications are not possible by the techniques used; however, it can be stated that the I and i antigens are readily detected both on glycoproteins and glycolipid extracts of erythrocytes, and SSEA-1 is detectable on those of granulocytes and HL 60 cells. However, in teratocarcinoma cells of the mouse these antigens are detected predominantly on glycoproteins.

Conclusions

Thus we are dealing with an interrelated system of carbohydrate antigens which includes the major blood group antigens and whose expression changes during successive stages of embryogenesis, differentiation and oncogenesis. The changes in the expression of the blood group antigens in various organs of the human foetus at different stages of development have been reviewed by Szulman (1980).

Our observations with monoclonal antibodies as well as studies from a number of other laboratories are reinforcing the concept that saccharides of the cell surface are important tumour-associated and differentiation antigens. However, none of the antigens thus far characterized has been uniquely specific for a given cell type or tumour. The changes we are observing seem to represent changes in the absolute amounts and the relative proportions of various carbohydrate structures, whose degree of expression in growing and differentiating cells is precisely programmed and genetically predictable. The changes in the carbohydrate structures may represent changes in (a) the availability of donor substrates, (b) the activities of glycosyltransferases or (c) the genes that code for the biosynthesis of these enzymes. The relationship of these changes to the neo-

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plastic process is unclear. The functions of the saccharide structures and their contributions to the properties of normal and abnormal behaviour of tumour cells are also not understood and will be the subject of future studies.

These glycosylation changes would be difficult to detect without the aid of monoclonal antibodies. Thus while the majority of these monoclonal antibodies may be inappropriate for the purpose for which they were raised, e.g. tumour diagnosis or immunotherapy (Feizi, 1983c), they are of unquestioned value as precise biochemical and immunological tools. Through the use of well-characterized monoclonal antibodies it can be anticipated that important advances will be made in the biochemistry of the neoplastic differentiation processes.


Surface glycoprotein changes during normal and malignant haematopoietic differentiation

CARL G. GAHBBERG,* LEIF C. ANDERSSON,† KIMMO K. KARHI* and HANS ROD†
*Department of Biochemistry, University of Helsinki, Unioninkatu 35, Helsinki, Finland, †Department of Pathology, University of Helsinki, Haartmanninkatu 3, Helsinki, Finland and ‡Institut für Hämatologie, Abteilung Immunologie der GSF, München, Federal Republic of Germany

Throughout life there is a continuous formation of the different types of human blood cells in the bone marrow and lymphoid organs. The cells originate from a common stem cell and differentiate along separate pathways to the final mature cells. During the differentiation they acquire their characteristic morphologies according to which the major types of cells originally were named. Using various antisera and other markers it has, however, become apparent that the cells can be subtyped into several additional groups.

The most functionally interesting cell surface molecules comprising various antigens, receptors and ‘markers’ characteristic of the cellular subsets are proteins and glycolipids (Gahmberg & Anderson, 1982a,b). Most of these proteins are glycoproteins (Gahmberg, 1976) with the carbohydrate exposed to the external milieu (Singer & Nicolson, 1972; Gahmberg & Hakomori, 1973; Steck & Dawson, 1974). We believe that to be able to eventually understand how the haematopoietic cell surface functions, it is necessary to characterize it at the molecular level. We have approached this problem by first developing methods to radioactively label the carbohydrate portions of cell-surface glycoproteins and glycolipids. Using the enzyme galactose oxidase, cell surface-located peracetylated N-acetylglalactosaminyl residues of glycoproteins and glycolipids can specifically be oxidized (Gahmberg & Hakomori, 1973). Alternatively cell-surface sialic acids are specifically oxidized using a low concentration of periodate at 0°C (Gahmberg & Andersson, 1977). The generated aldehyde groups are subsequently reduced