The carbohydrate specificities of the monoclonal antibodies 29.1, 455 and 3C1B12 to the epidermal growth factor receptor of A431 cells

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Sixteen hybridoma-derived antibodies to the epidermal growth factor receptor of A431 cells were studied with respect to their reactions with blood group-related carbohydrate structures. Twelve of these were assessed as recognizing carbohydrate determinants on the basis of their immunostaining of reference blood group substances on nitrocellulose paper. Three of these antibodies were further investigated by inhibition of binding assays with glycoproteins and structurally defined oligosaccharides or by haemagglutination of erythrocytes before and after treatment with endo-β-galactosidase. Two of the antibodies, 29.1 and 455, were shown to have blood group A-related specificities which differed from one another and from those of monoclonal anti-A antibodies described previously. The third antibody, 3C1B12, which was shown to recognize a determinant based on αL3 fucosylated Type 2 chains on linear and branched backbone sequences, also differs from previously described monoclonal antibodies of 3-fucosyl-N-acetyllactosamine type, such as anti-SSEA-1 (anti-mouse embryo) and several antibodies to human myeloid cells. While these antibodies are invaluable in providing structural information on the carbohydrate chains of the receptor glycoprotein and should help to elucidate their functions, their use as 'anti-receptor' reagents in cell biology will be influenced by the knowledge that the determinants they recognize are shared by other glycoproteins and glycolipids of diverse cell types.
Monoclonal antibodies are being widely used in studies of differentiation and tumour-associated antigens and receptors for various ligands on normal and neoplastic cells. Recent studies (1,2) have established that even when a single glycoprotein component is immune-precipitated by a monoclonal antibody, the antigenic determinant recognized may be a carbohydrate structure shared by other glycoproteins and glycolipids on different cell types and in secretions. Thus, knowledge of the precise determinants recognized by such antibodies is crucial for the correct interpretation of their reaction patterns and their rational applications in cell biology.

A considerable number of monoclonal antibodies have been raised against the epidermal growth factor (EGF) receptor of the epidermoid carcinoma cell line A431. Three of these have been studied in detail and they recognize blood group-related carbohydrate structures; two recognize determinants related to blood group A (1,3) and the third, a blood group H-related structure (4). In the present studies we have investigated 16 additional hybridoma antibodies raised in three different laboratories against the EGF receptor of A431 cells and have evidence for carbohydrate specificity in 12 of them. The detailed characterization of three of these antibodies is the subject of the present report. These are antibodies 29.1 (ref. 5), 455 (ref. 6) and 3C1B12 (present report).

Materials and Methods

Monoclonal antibodies

Sixteen mouse-hybridoma-derived monoclonal antibodies to the EGF receptor of the A431 cell line were studied. These were antibody 29.1 raised against paraformaldehyde-fixed A431 cells (5) and ten antibodies that were selected from three separate immunization-fusion procedures (5) using purified EGF receptor: (i) 3CIB12; (ii) 5B7A12, 5B7B11, 5C3C5, 5C3A4(2), 5C3C6(2) and (iii) 6B10D2, 6B10B11, 6B10C10 and 6B10G6. Antibody 528 (7) and antibodies 455, 225 and 579 (6) were selected from four different immunization-fusion procedures using partially purified EGF receptor. Antibody EGR-1 raised against trypsinized A431 cells (8) was a gift of Dr. P. Goodfellow, Imperial Cancer Research Fund, Lincolns Inn Fields, London. These antibodies were designated as having anti-receptor specificities on the basis of their ability to inhibit the binding of EGF (antibodies 225, 528 and 579) or precipitate the EGF receptor from solubilized A431 cells. Antibodies 29.1, 455, 225, 528 and 579 were used as purified immunoglobulins; EGR-1 as culture supernatant and the remaining antibodies were used as ascites.

Two human sera, Den and MaT, containing monoclonal anti-i and anti-I antibodies, respectively, have been described previously (9).

Glycoproteins and erythrocytes

Five glycoproteins from ovarian cysts (gifts from Dr. E. A. Kabat, Columbia Medical Centre, New York and Dr. W. M. Watkins, Clinical Research Centre, Harrow) and two from human meconium were used as reference blood group substances. Their designations and blood group activities are given in Table 1 and their full citations given previously (1,10).
Human erythrocytes of blood groups O, A and B from healthy adults were stored in anticoagulant citrate-dextrose for up to one week; erythrocytes from a blood group A donor of i antigen type were stored in glycerol at -20°C for two years and reconstituted as described by Mollison (11).

**Oligosaccharides**

N-acetylgalactosamine was from Sigma (Poole, U.K.), the blood group A-active trisaccharide with spacer arm, designated A-trisaccharide-O-S (Fig. 1) and synthesized chemically (12) was a gift of Dr. C. Kolar, Research Laboratories of Behringwerke AG, Marbourg, Germany; the A tri- to heptasaccharides were isolated from human urine (gifts of Dr. G. Strecker, University of Lille, Unité de Science et de Technique, Lille, France) or the faeces of breast-fed children (gifts of Drs. A. Lundblad and H. Sabharwal, University Hospital Lund, Sweden); their full citations have been given previously (1). The A-like disaccharide-O-S and A-like trisaccharide were obtained from the A-trisaccharide-O-S and A-tetrasaccharide, respectively, by mild-acid hydrolysis (0.02 M H₂SO₄, 100°C; hydrolysis time 90 min and 150 min respectively). The extent of hydrolysis of the oligosaccharides, as assessed by thin layer chromatography, was greater than 90%. The chemically synthesized trisaccharide, 3-fucosyl-N-acetylgalactosamine (designated 3F in Table 2) was a gift of Dr. P. Sinay, University of Orleans, France. The milk oligosaccharide N₂ was a gift of Dr. T. Rohr (Abbot Laboratories, North Chicago, U.S.A.). Lacto-N-fucopentaose III (LNF III) and LNF II were isolated (13) from a fraction of milk oligosaccharides (gift of Dr. J. P. Prieels, University of Brussels, Belgium).

**Immunological techniques**

Immunostaining of glycoproteins on nitrocellulose paper (1) was carried out using 10 µg/ml of the purified antibodies, 1:100 dilution of the ascites and undiluted culture supernatant. Negative controls were normal mouse immunoglobulins (Pel-Freeze, Middlesex, U.K.), normal mouse serum 1:100 dilution or culture medium containing 10% foetal calf serum.

Solid phase radioimmunoassay using 29.1 and 455 antibodies and the reference blood group A-active glycoproteins MSS coated onto polyvinyl plates was an adaptation (3) of the method of Cobbold and Waldman (14).

Liquid phase, double antibody radioimmunoassay using antibody 3C1B12 and 125I-labelled meconium glycoproteins was performed as described previously (15,16).

Haemagglutination (17) was carried out at 4°C with untreated erythrocytes or with erythrocytes which were treated with endo-β-galactosidase of *Bacteroides fragilis* (18,19) as described previously (20).

**Results**

**Preliminary screening for blood group related specificities**

On the basis of initial nitrocellulose-immunostaining, using reference blood group substances isolated from human ovarian cysts and
meconium, the 16 antibodies to the EGF receptor of A431 cells could be divided into 3 groups: (a) those giving immunostaining of glycoproteins with blood group A or A-like activity but not those lacking this activity; these were antibodies 29.1 and 455; (b) those giving moderate or strong immunostaining of glycoproteins from non-secretor individuals of Lewis-positive or negative types, variable immunostaining with blood group A, B, H and Le\(^B\)-active glycoproteins from secretors, but no reactions with mild-acid-treated glycoproteins depleted of fucose residues; these were antibodies 3C1B12, 5B7A12, 5B7B11, 5C3C5, 5C3A4(2), 5C3C6(2), 6B10D2, 6B10B11, 6B10C10 and 6B10G6, and (c) those giving no immunostaining with the reference blood group substances, 225, 528, 579 and EGR-1.

Antibodies 29.1, 455 and one representative member of the second group of antibodies, 3C1B12, were selected for further investigation.

**Antibodies 29.1 and 455**

Both antibodies bound to the reference blood group A substance MSS in polyvinyl plate binding assays (results not shown).

The binding of antibody 29.1 was best inhibited by the Type 2 based blood group A-tetrasaccharide and the A-like trisaccharide derived from it by mild acid treatment [0.11 and 0.12 nmol gave 50% of inhibition of binding respectively (Fig. 1A)]. The difucosylated A-pentasaccharide was only slightly less active (0.22 nmol gave 50% inhibition). The A-like disaccharide-O-5 and A-trisaccharides were 8 times less active, and those containing the Type 1 based mono- or difucosylated blood group A sequences, A-hexasaccharide and A-heptasaccharide, were 10 times less active than the Type 2 based structures. The monosaccharide N-acetylgalactosamine was 2500 times less active. We conclude that the combining site of this antibody preferentially reacts with the Type 2 based blood group A-related structure. The presence of the \(\alpha1+2\) linked fucose residue does not enhance reactivity and the presence of the second \(\alpha1+3\) linked fucose residue marginally impairs reactivity with this antibody.

The oligosaccharide inhibition data with 455 antibody (Fig. 1B) showed that it also recognizes the Type 2 based blood group A structure. However, among the oligosaccharides tested, only the monofucosylated A-tetrasaccharide gave inhibition. In marked contrast to the findings with antibody 29.1, neither the difucosylated Type 2 blood group A structure nor the other blood group A oligosaccharides gave inhibition at the highest concentrations tested. Thus, the presence of the \(\alpha1+2\) linked fucose residue is required for reaction with 455 antibody, and the second \(\alpha1+3\) linked fucose residue masks the reactivity with this antibody.

The amount of A-tetrasaccharide (4 nmol) required for 50% inhibition of antibody 455 is 5-24 times higher than the amounts of the most optimal oligosaccharides required by antibody 29.1 (0.11-0.12 nmol) and two previously characterized anti-A antibodies (0.6-0.7 nmol). This raised the possibility that 455 antibody might recognize an additional structure on either a linear or a branched backbone sequence. Since oligosaccharides with such structures were not available for testing in inhibition assay, we investigated this possibility by performing haemagglutination studies with blood group A erythrocytes of i and I antigen types which are rich in linear and branched
Fig. 1. Inhibition of binding of anti-EGF receptor antibodies 29.1 (A) and 455 (B) to the reference blood group A substance, MSS. The binding of antibodies 29.1 and 455 to the blood group A substance was measured by solid phase radioimmunoassay and inhibition of binding assays were performed at 0.003 µg/ml and 0.03 µg/ml concentration of the antibodies, respectively, as described in the Materials and Methods section. Symbols for oligosaccharides and their structures are as follows:

- △ N-acetylgalactosamine: GalNAc
- ● A-like disaccharide-0-S: GalNAc1+3Galβ1-0-(CH_2)_2-NH-CO(CH_2)_7COOCH_3
- ○ A-trisaccharide-0-S: GalNAc1+3Galβ1-0-(CH_2)_2-NH-CO(CH_2)_7COOCH_3 +1,2
- ○ A-trisaccharide: GalNAc1+3Gal
- ○ A-like trisaccharide: GalNAc1+3Galβ1+4Glc
- ▪ A-tetrasaccharide: GalNAc1+3Galβ1+4Glc +1,2
- ▼ A-pentasaccharide: GalNAc1+3Galβ1+4Glc +1,2 +1,3
- ■ A-hexasaccharide: GalNAc1+3Galβ1+3GlcNacβ1+3Galβ1+4Glc +1,2
- ◆ A-heptasaccharide: GalNAc1+3Galβ1+3GlcNacβ1+3Galβ1+4Glc +1,2 +1,4
Type 2 backbone sequences, respectively (9,21) such as those shown below.

\[
\begin{align*}
\text{Fuc}^\alpha & \downarrow_{1,2} \\
\text{GalNAc}\alpha & +3\text{Gal}\beta & +4\text{GlcNAc}\beta & +3\text{Gal}\beta & +4\text{Glc}/\text{GlcNAc}
\end{align*}
\]

Antibody 29.1 was also tested for comparison. Both antibodies gave the same haemagglutination titre with group A erythrocytes of i and I antigen types; minimum agglutinating concentration with both antibodies was approx. 0.3 µg/ml with erythrocytes of blood group AI and Ai (neither agglutinated erythrocytes of blood groups O and B). The haemagglutination of AI and Ai erythrocytes by these antibodies was not affected by treatment of the erythrocytes by endo-β-galactosidase, which cleaves the linear domains of these long chain backbone structures at internal Galβ1→4GlcNAc linkages (18). In control experiments, haemagglutination by anti-i antibody Den was reduced from 1:32 000 to less than 1:50 and haemagglutination by anti-I antibody Ma was reduced from 1:32 000 to 1:800 after treatment of the Ai and AI erythrocytes, respectively, with this enzyme. These observations suggest that neither antibody distinguishes between the linear and branched backbone sequences. Nor do they have a requirement for long chain Type 2 (poly-N-acetyllactosamine) structures. However, these studies do not rule out the possibility that antibody 455 recognizes a part of the GlcNAcβ1→3Gal backbone sequence as found on the glycolipid structure for it is known that on intact erythrocytes, the Galβ1→4Glc linkage near the lipid moiety resists cleavage by endo-β-galactosidase (22).

\[
\begin{align*}
\text{GalNAc}\alpha & +3\text{Gal}\beta & +4\text{GlcNAc}\beta & +3\text{Gal}\beta & +4\text{Glc}/\text{GlcNAc}
\end{align*}
\]

\[
\text{Fuc}^\alpha & \downarrow_{1,2} \\
\text{GalNAc}\alpha & +3\text{Gal}\beta & +4\text{GlcNAc}\beta & +3\text{Gal}\beta & +4\text{Glc}/\text{GlcNAc}
\]

3C1B12 antibody

In soluble phase radioimmunoassays this antibody resembled anti-SSEA-1 in binding to 125I-labelled meconium glycoproteins (results not shown) and this binding was inhibited by reference glycoproteins of non-secretor type (Table 1). As with anti-SSEA-1, the antigen involved is acid labile, for there was no inhibition with mild-acid-treated meconium glycoproteins. A difference between the two antibodies was that the reference blood group A-active glycoprotein and, to a lesser extent, the HLeb active glycoprotein were inhibitory with 3C1B12 antibody whereas they gave little inhibition with SSEA-1.
Table 1. Inhibition of binding of anti-EGF receptor antibody 3C1B12 by glycoproteins with known blood group-related antigen activities

The binding of antibody 3C1B12 to radiolabelled meconium glycoproteins was measured by soluble phase radioimmunoassay, and inhibition of binding was performed at 1:10 000 dilution of ascites as described in the Materials and Methods section. Results with the antit-mouse embryo antibody, anti-SSEA-1, taken from a previous study (16) are included for comparison.

<table>
<thead>
<tr>
<th>Glycoprotein (known antigen activities)</th>
<th>Antibody</th>
<th>( \mu g/ml ) giving 50% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meconium, non-secretor type (Ii, SSEA-1, Le( ^a ), A-like)</td>
<td>3C1B12</td>
<td>2.8</td>
</tr>
<tr>
<td>Meconium, mild acid treated (Ii, A-like)</td>
<td>3C1B12</td>
<td>—*</td>
</tr>
<tr>
<td>F1 (Ii, SSEA-1)</td>
<td>3C1B12</td>
<td>22</td>
</tr>
<tr>
<td>N-1 (I, SSEA-1, Le( ^b ))</td>
<td>3C1B12</td>
<td>6.8</td>
</tr>
<tr>
<td>JS (H, Le( ^b ))</td>
<td>3C1B12</td>
<td>70</td>
</tr>
<tr>
<td>MSS (A)</td>
<td>3C1B12</td>
<td>5</td>
</tr>
<tr>
<td>Beach (B)</td>
<td>3C1B12</td>
<td>—</td>
</tr>
</tbody>
</table>

*Inactive at highest level tested, 100 \( \mu g/ml \).

+Percent inhibition at highest level tested, 100 \( \mu g/ml \).

Inhibition assays with oligosaccharides (Table 2) showed that 3C1B12 antibody, like anti-SSEA-1, was strongly inhibited by the Type 2 based milk oligosaccharide LNF III (2 and 0.9 nmol of oligosaccharide were required, respectively, for 50% inhibition) but not by the Type 1 based isomer LNF II. However, 3C1B12 antibody differed from anti-SSEA-1 in that it was poorly inhibited by the trisaccharide, 3F (31% inhibition was obtained with 19 nmol, compared with 50% inhibition at 0.58 nmol with anti-SSEA-1). Another major difference was that the inhibitory activity of oligosaccharide N2 with 3C1B12 antibody was comparable to that of LNF III, whereas with anti-SSEA-1 the former oligosaccharide had little inhibitory activity (32% inhibition at 8 nmol). From these studies we deduce that the combining site of 3C1B12 requires additional backbone sequence consisting of at least a \( \beta 1\rightarrow3 \) or 6Gal linkage as shown below:

\[
\begin{align*}
\text{Gal} & \rightarrow 4 \text{GlcNAc} \beta 1^* \\
& \rightarrow 1,3 \\
& \text{Fuc} \alpha
\end{align*}
\]
Table 2. Inhibition of binding of anti-EGF receptor antibody 3C1B12 and the anti-embryo antibody, anti-SSEA-1, by oligosaccharides

Soluble phase inhibition assays were performed as described in the Materials and Methods section. The inhibition data with anti-SSEA-1 and oligosaccharides LNF II and LNF III are taken from a previous study (13).

<table>
<thead>
<tr>
<th>Oligosaccharide</th>
<th>Antibody</th>
<th>3C1B12</th>
<th>Anti-SSEA-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>3F</td>
<td>Galβ1+4GlcNAc</td>
<td></td>
<td>(31%)+</td>
</tr>
<tr>
<td></td>
<td>Fuca</td>
<td></td>
<td>0.58</td>
</tr>
<tr>
<td>LNF III</td>
<td>Galβ1+4GlcNAcβ1+3Galβ1+4Glc</td>
<td>2</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Fuca</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Galβ1+3GlcNAcβ1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fuca</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>6Galβ1+4Glc</td>
<td>4.5</td>
<td>(32%)</td>
</tr>
<tr>
<td></td>
<td>Fuca</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LNF II</td>
<td>Galβ1+3GlcNAcβ1+3Galβ1+4Glc</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fuca</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Percent inhibition at the highest level tested. 19 nmol (3F) and 8 nmol (N2).
*No inhibition at the highest level tested, 12 nmol.

Discussion

General remarks

These studies show that a high proportion of hybridoma antibodies raised against the EGF receptors of A431 cells recognize carbohydrate structures. Of the 16 antibodies investigated in the present study, 12 were of this category. The remaining 4 antibodies did not react with the reference blood group substances, and were not investigated further, but may recognize determinants expressed on other types of carbohydrate structures (and substituents associated with them) or on the polypeptide moiety of the receptor glycoprotein.

We have previously given evidence (23) for the presence of oligosaccharides with long-chain backbone sequences of lacto/neolacto type bearing a variety of blood group-related carbohydrate structures. The present work, together with the previous studies on the blood group related specificities of monoclonal antibodies to the EGF receptor of this cell line, show that these structures are strongly antigenic components of the receptor glycoprotein. Since the EGF receptor and its degradation fragments are the main components that
are immune-precipitated by these antibodies from membranes of A431 cells, it can be deduced that this receptor is the major blood group-active glycoprotein on the surface of A431 cells.

Carbohydrate determinants recognized by the anti-EGF receptor antibodies

The five antibodies that we have characterized in detail, in the present and previous (1,3) studies, all differ in the carbohydrate epitopes they recognize, as shown below:

TL5 \[\text{GalNAc} \alpha 1 \rightarrow 3 \text{Gal} \beta 1 \rightarrow 3/4 \text{GlcNAc} \]
\[\begin{array}{c}
\uparrow 1,2 \\
\text{Fuca}
\end{array}\]

EGR/G49 \[\text{GalNAc} \alpha 1 \rightarrow 3 \text{Gal} \beta 1 \rightarrow 3/4 \text{GlcNAc} \]
\[\begin{array}{c}
\uparrow 1,2 \quad \uparrow 1,4/3 \\
\text{Fuca} \quad \text{Fuca}
\end{array}\]

29.1 \[\text{GalNAc} \alpha 1 \rightarrow 3 \text{Gal} \beta 1 \rightarrow 4 \text{GlcNAc} \]
\[\begin{array}{c}
\uparrow 1,2 \quad \uparrow 1,3 \\
\pm \text{Fuca} \quad \pm \text{Fuca}
\end{array}\]

455 \[\text{GalNAc} \alpha 1 \rightarrow 3 \text{Gal} \beta 1 \rightarrow 4 \text{Glc/GlcNAc[} \beta 1-3 \text{Gal}] \]
\[\begin{array}{c}
\uparrow 1,2 \\
\text{Fuca}
\end{array}\]

3CIB12 \[\text{Gal} \beta 1 \rightarrow 4 \text{GlcNAcB} \]
\[\begin{array}{c}
\uparrow 1,3 \\
\text{Fuca}
\end{array}\]

It is not yet established whether the requirement for a relatively high level of the A-tetrasaccharide structure for inhibition of antibody 455 reflects a preference for an N-acetylgalcosamine rather than a glucose residue or even the requirement for additional sequence(s) in the backbone region. It should be noted that monoclonal antibody 29.1 and the other anti-A monoclonal antibody, TL5, can react with the GalNAc\(\alpha 1 \rightarrow 3\)Gal sequence in the absence of the blood group H-associated \(\alpha 1 \rightarrow 2\) linked fucose residue. From this, it can be predicted that these two antibodies would react with cells derived from individuals lacking the blood group A gene-specified N-acetylgalactosaminyltransferase but containing another N-acetylgalactosaminyltransferase which can use the Type 1 or 2 backbone structures directly (the blood group A gene-specified enzyme requires the blood group H structure as an acceptor substrate [24]). A cellular enzyme of this type has not yet been described to our knowledge, but is by no means ruled out (W. M. Watkins, personal communication). Thus, antibodies TL5 and 29.1 may react with certain cells that are derived from persons lacking the blood group A gene.

Antibody 3CIB12 which recognizes \(\alpha 1 \rightarrow 3\) fucosylated Type 2 chains differs from the previously described hybridoma antibody, anti-SSEA-1, which recognizes an 8-cell-stage specific antigen of the mouse embryo, in its lack of inhibition with the trisaccharide 3-fucosyl-N-acetyllactosamine and its strong inhibition with the branched oligosaccharide,
N2. Another group of hybridoma antibodies with specificities involving the 3-fucosyl-N-acetyllactosamine sequence are those which recognize granulocytes among cells of the human peripheral blood (10,25). In unpublished experiments, we (H.C.G. and T.F.) have shown that several of the anti-granulocyte antibodies require 4-10-fold or greater concentrations of the branched oligosaccharide N2 than of LNF III for 50% inhibition. Thus, antibodies against the 3-fucosyl-N-acetyllactosamine containing carbohydrate sequences differ in their ability to react with oligosaccharides containing this structure B1†6 linked to a galactose residue. The reaction of 3C1B12 antibody with the reference glycoproteins JS and MSS, contrasting with a lack of reaction with anti-SSEA-1 and the anti-neutrophil antibodies, may be due to the presence of the determinant on branched B1†6 linked carbohydrate backbones in these two glycoproteins.

Biological relevance of the saccharide antigens of the receptor glycoproteins

Among the anti-EGF receptor antibodies, only three, namely 225, 528 and 579 (ref. 6) effectively compete with the binding of EGF to cells and they inhibit the metabolic effects of the growth factor. These antibodies, which have related specificities (each competes with the binding of the others) are likely to be directed at antigenic determinants that are in or close to the EGF binding site on the receptor (6). However, they were not among antibodies that bound to the reference blood group glycoproteins. Thus, there is no evidence thus far that blood group related structures are directly involved in an interaction with the growth factor. However, observations with two of the anti-A-related antibodies, TL5 and EGR/G49, indicate that perturbation of the carbohydrate structures they recognize may influence cell growth. Treatment of human foreskin fibroblasts with TL5 antibody followed by cross-linking of the bound antibody with anti-mouse immunoglobulins has been reported to stimulate uptake of $^3$H thymidine into the fibroblasts (26). EGR/G49 antibody has been reported to inhibit the growth of A431 cells (27). These observations raise the possibility (28) that blood group A or other carbohydrate structures on the receptor glycoprotein may be receptors for endogenous ligands (e.g. lectins) that are distinct from EGF and may mediate growth regulatory effects in their own right. There is so far no direct evidence that endogenous lectins function as growth regulators by interacting with receptors for growth factors. However, there is a distinct possibility that a widely distributed soluble, β-galactoside-binding lectin of animal tissues (29) may have such a role. Not only are the levels of lectin developmentally regulated (29) but, in studies which will be described elsewhere, we (S. R. Carding, T. Feizi and colleagues) have observed increased levels of a family of proteins antigenically related to β-galactoside-binding lectin in transformed and mitogen stimulated lymphocytes (30). And, in experiments in which $^{125}$I-labelled lectin was overlaid onto cell lysates which had been electrophoresed in SDS polyacrylamide gels and electrotransferred onto nitrocellulose, we have detected several potential receptors for lectin; these behave as glycoproteins with apparent molecular masses in the range 50-130 kDa in cultured fibroblasts derived from skin, and 10-180 kDa in bovine gastric mucosa. Further investigations are now required
to establish whether the EGF receptor, which contains carbohydrate chains with \( \beta \)-galactoside termini (23), interacts with this and other endogenous lectins, in-vivo.

It can be envisaged that these various anti-carbohydrate antibodies will be invaluable reagents in studies of the structures and roles of the carbohydrate chains of the EGF receptor and those of other glycoproteins and glycolipids. Such antibodies have already enabled considerable inferences to be made about the backbone and peripheral region structures of the carbohydrate chains of the EGF receptor of A431 cells (23). On the other hand, great caution is required in the use and interpretation of the reactions of these monoclonal 'anti-receptor' antibodies with whole cells. Their cellular reaction patterns will be influenced by the presence of glycolipids or other minor glycoproteins with similar carbohydrate chains which may not be detected by immune precipitation. Moreover, when such antibodies are used for the isolation of the EGF receptor from cell lysates, there will be a selective enrichment of receptor subpopulations with a high density of a particular carbohydrate side chain. The same principles will apply to monoclonal antibodies against other glycoprotein receptors.

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References