*Carbohydrate Research*, 141 (1985) 347–349
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

## Note

# Monoclonal antibody 101 that precipitates the glycoprotein receptor for epidermal growth factor is directed against the Y antigen, not the H type 1 antigen

JACQUES LE PENDU*, PAM FREDMAN†, NANCY D. RICHTER‡, JOHN L. MAGNANI†, MARK C. WILLINGHAM‡, IRA PASTAN‡, RAFAEL ORIOL*, AND VICTOR GINSBURG†

**Institut d'Immuno-Biologie, Hôpital Broussais, F-75674 Paris (France); †National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20205 (U.S.A.); and ‡National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205 (U.S.A.)*

(Received October 26th, 1984; accepted for publication, January 30th, 1985)

Monoclonal antibody 101 produced by a hybridoma obtained by fusion with NS-1 myeloma cells of spleen cells from a mouse immunized with the human epidermoid carcinoma cell line, A431, specifically precipitates epidermal growth factor receptor, a glycoprotein of 170 000 $M_r$ solubilized from A431 cell membranes[1]. The antibody also binds to neutral glycolipids extracted from A431 cells, as evidenced by solid phase radioimmunoassay and by autoradiography. As the binding of the antibody to its targets was inhibited by lacto-*N*-fucopentaose I but not by 2'-fucosyllactose or related oligosaccharides, it was proposed that antibody 101 is probably directed against the human blood group H type 1 sugar sequence α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc . . . and that the epidermal, growth-factor receptor contains this sequence[2].

Subsequent immunohistological studies with antibody 101, however, indicated that it was directed against a type 2 antigen, not a type 1 antigen[3]. The specificity of antibody 101 was therefore reexamined and, as reported herein, found to be directed against the difucosyl type 2 sugar sequence of the Y antigen, α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-β-D-Glc*p*NAc . . . The inhibition of binding originally observed with lacto-*N*-fucopentaose I was due to impurities in the preparation used in the studies. Monoclonal antibodies directed against the Y antigen have been reported previously[4–7].

Thus, the epidermal growth factor receptor of A431 cells contains the Y antigen sugar sequence as well as the A- and Lewis-antigen sugar sequences[8–10].

## EXPERIMENTAL

*General methods.* — Antibody specificity was determined with artificial antigens produced by linking oligosaccharides to bovine serum albumin *via* an

0008-6215/85/$ 03.30 

TABLE I

STRUCTURE OF ARTIFICIAL ANTIGENS

| *Antigen specificity* | *Structure*[a] |
|---|---|
| Type 1 precursor | β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc→OR |
| Type 2 precursor | β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc→OR |
| H type 1 | α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc→OR |
| H type 2 | α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc→OR |
| Le[a] | β-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→4)]-β-D-Glc*p*NAc→OR |
| X | β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-β-D-Glc*p*NAc→OR |
| Le[b] | α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→3)-[α-L-Fuc*p*-(1→4)]-β-D-Glc*p*NAc→OR |
| Y | α-L-Fuc*p*-(1→2)-β-D-Gal*p*-(1→4)-[α-L-Fuc*p*-(1→3)]-β-D-Glc*p*NAc→OR |
| A type 1 | α-D-Gal*p*-NAc-(1→3)-[α-L-Fuc*p*-(1→2)]-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc→OR |
| A type 2 | α-D-Gal*p*-NAc-(1→3)-[α-L-Fuc*p*-(1→2)]-β-D-Gal*p*-(1→4)-β-D-Glc*p*NAc→OR |

[a] R = $[(CH_2)_8CONH]_x$ (bovine serum albumin)

aliphatic linking arm[4,11], and by hapten inhibition studies[2]. The oligosaccharides used for linking were synthetic and were gifts from Dr. R. U. Lemieux and Chembiomed Ltd. (Edmonton, Canada).

*Specificity studies.* — A solid-phase radioimmunoassay was developed to measure the binding of antibody 101 to the artificial antigens listed in Table I. Polystyrene tubes (L.E.F. Viry-Chatillon, France) were coated with artificial antigen diluted to 10 μg/mL in PBS by incubating overnight at room temperature. Purified antibody 101 was added in two-fold serial dilutions. Binding was measured by the sequential addition of rabbit anti-mouse immunoglobulins and $^{125}$I-labelled protein A. Bovine serum albumin (3%) was used to prevent nonspecific binding. Antibody 101 bound only to the Y antigen and not to the other antigens tested (Fig. 1).

The specificity of antibody 101 for the Y antigen sugar sequence was confirmed by solid-phase radioimmunoassay using microtiter plates and by hapten in-

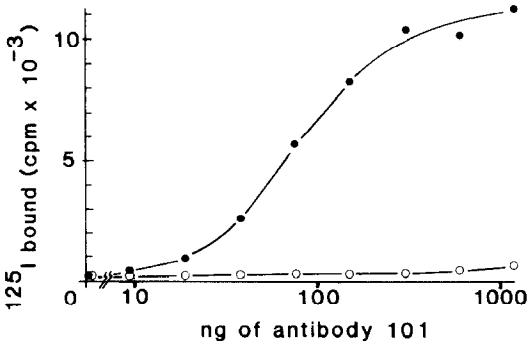


Fig. 1. Binding of antibody 101 to artificial antigens. Binding was measured by solid-phase radioimmunoassay as described in the Experimental section: (●——●) Y antigen; (○——○) other antigens listed in Table I.

hibition as previously described[2]. The antibody bound specifically to purified Y-active glycolipid (kindly supplied by Dr. S. Hakomori, Seattle) and this binding was specifically inhibited by synthetic Y-active tetrasaccharide (kindly supplied by Dr. P. Sinaÿ, Orléans, France). Fifty percent inhibition was obtained with 0.02mM tetrasaccharide. Lacto-*N*-fucopentaose I at 0.5mM concentration did not inhibit binding.

REFERENCES

1 N. D. RICHERT, M. C. WILLINGHAM, AND I. H. PASTAN, *J. Biol. Chem.*, 258 (1983) 8902–8907.
2 P. FREDMAN, N. D. RICHERT, J. L. MAGNANI, M. C. WILLINGHAM, I. PASTAN, AND V. GINSBURG, *J. Biol. Chem.*, 258 (1983) 11206–11210.
3 R. MOLLICONE, J. BARA, J. LE PENDU, AND R. ORIOL, *Lab. Invest.*, 53 (1985) in press.
4 C. BLAINEAU, J. LE PENDU, D. ARNAUD, F. CONNAN, AND P. AVNER, *EMBO J.*, 2 (1983) 2217–2222.
5 K. ABE, J. M. MCKIBBIN, AND S. HAKOMORI, *J. Biol. Chem.*, 258 (1983) 11793–11797.
6 A. BROWN, T. FEIZI, H. C. GOOI, M. J. EMBLETON, J. K. PICARD, AND R. W. BALDWIN, *Biosci. Rep.*, 3 (1983) 163–170.
7 K. O. LLOYD, G. LARSSON, N. STROMBERG, J. THURIN, AND K.-A. KARLSSON, *Immunogenetics (N.Y.)*, 17 (1983) 537–541.
8 P. J. PARKER, S. YOUND, W. J. GULLICK, E. L. V. MAYES, P. BENNETT, AND M. D. WATERFIELD, *J. Biol. Chem.*, 259 (1984) 9906–9912.
9 H. C. GOOI, J. SCHLESSINGER, I. LAX, Y. YARDEN, T. A. LIBERMANN, AND T. FEIZI, *Biosci. Rep.*, 3 (1983) 1045–1053.
10 R. A. CHILDS, M. GREGORIOU, P. SCUDDER, S. J. THORPE, A. R. REES, AND T. FEIZI, *EMBO J.*, 3 (1984) 2227–2233.
11 R. U. LEMIEUX, *Chem. Soc. Rev.*, 7 (1978) 423–452.