Carbohydrate Research, 178 (1988) 333–336
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Analysis of a mouse monoclonal antibody detecting the H type 1 blood group determinant*,§

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(Received July 28th, 1986; accepted for publication, September 4th, 1986)

Application of the hybridoma procedure[1] has resulted in the production of an extensive series of mouse monoclonal antibodies (moAbs) directed against the A, B, H, Lewis family of blood group determinants[2–4]. Some of these antibodies were generated by deliberate immunization of mice with blood group-active antigens, but the majority resulted from immunizations with more complex immunogens such as human tumor cells. These antibodies provide a powerful collection of reagents for the analysis of blood group structures and their distribution in tissues. Of the 16 determinants that make up this family[4], moAbs to all of the structures, except to some of the B variants and to H type 1, are now available. We now report an antibody specific for the monofucosyl H type 1 determinant.

Immunization of an NZB mouse (*cf.* ref. 5) with the human colonic cancer cell line SW403, and subsequent fusion of splenocytes with the mouse myeloma cell line SP 2/0 resulted in the isolation of a number of clones producing antibody to the immunizing cell line. These antibodies were screened on tumor cell lines by use of a red cell rossetting assay with protein A indicator cells in an attempt to isolate IgG3-producing hybridomas. One of the reactive clones was shown to react with a blood group H glycoprotein, and not with A, B, Le^a, or precursor glycoproteins. This hybridoma was subcloned twice and was designated 17-206; it produces an antibody of the IgG3 sub-class.

Further analysis of this antibody showed that it did not agglutinate A, B, or O erythrocytes (from either Le^{a+} or Le^{b+} individuals), in contrast to an anti-Y (Le^y) antibody (F-3[6]) which agglutinated O erythrocytes preferentially. Examina-

*Dedicated to Professor Walter T. J. Morgan.

§This work was supported by grants from the National Cancer Institute (CA-08478 and CA-33049), National Institutes of Health, and the Swedish Medical Research Council.

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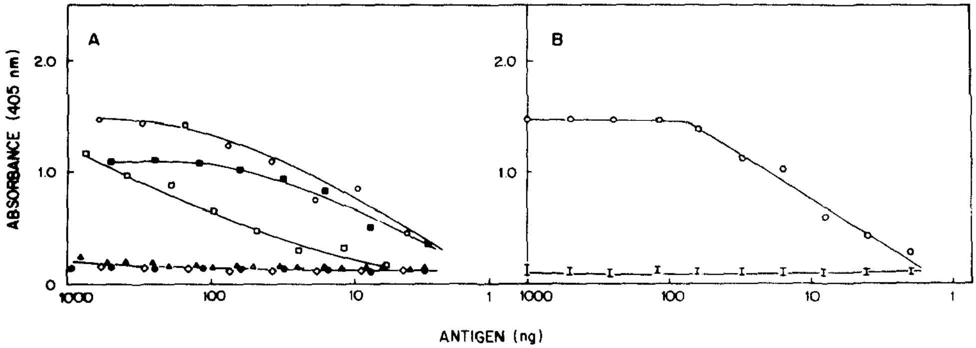

Fig. 1. (A) Reactivity of Ab 17-206 with blood group glycoproteins in an ELISA: (○) H glycoprotein (H500), (□) HLe^b glycoprotein (Tighe), (■) HLe^b glycoprotein (H116), (▽) A glycoprotein (MSS), (◇) B glycoprotein (Beach), (◆) Le^a glycoprotein (N1), and (▲) precursor glycoprotein (OG). Antibody dilution: 1:100 of ascites fluid. (B) Reactivity of Ab 17-206 with blood group glycolipids in an ELISA: (○) H-1-5 (IV^{2}Fucα-Lc$_4$Cer), (I) range shown by other glycolipids tested: LNTCer (Lc$_4$Cer), LNneoTCer (nLc$_4$Cer), H-2-5 (IV^{2}Fucα-nLc$_4$Cer), Le^a (III^{4}Fucα-Lc$_4$Cer, X (III^{3}Fucα-nLc$_4$Cer, Le^b (III^{4}Fuc, IV^{2}Fucα-Lc$_4$Cer), and Y (III^{3}Fuc,IV^{2}Fucα-nLc$_4$Cer). Dilution of antibody: 1:100 of ascites fluid.

tion of the reactivity of Ab 17-206 with an extended series of blood group glycoproteins by an enzyme-linked immunosorbent assay (ELISA) showed that it reacted preferentially with an H glycoprotein from an O Le^{a-}, Le^{b-} secretor individual; some reactivity was also demonstrated with two HLe^{b+} glycoproteins, but no reaction was observed with A, B, Le^a, and precursor glycoproteins (Fig. 1A). Of a panel of eight blood group glycolipids, Ab 17-206 was reactive only with the H type 1 structure (Fig. 1B). The specificity of the antibody was confirmed by use of the immunostaining procedure[7] on glycolipids separated by t.l.c. Ab 17-206 was found to react with the H type 1 pentaglycosyl ceramide[8] (IV^2 Fucα-Lc$_4$Cer), which was distinguished from the H type 2 isomer, IV^{2}Fucα-nLc$_4$Cer (Fig. 2). Subsequent spraying of the t.l.c. plate with oricinol–sulfuric acid reagent showed that the antibody was reacting with the H type 1 glycolipid and not with a contaminant in the preparation. T.l.c. immunostaining was also used to confirm nonreactivity with H type 2, Y-, and Le^b- and Le^a-active glycolipids (data not shown). Ab 17-206 showed no reactivity with the neutral glycolipids isolated from O erythrocytes; this was in contrast to the anti-H type 2 antibody[9] H-11 which stained two components, presumably[10] H_1 and H_2 (Fig. 2).

We conclude that Ab 17-206 reacts specifically with the H type 1 blood group determinant in both glycoproteins and glycolipids. No reactivity with H type 2 or with Y (Le^y) structures was observed (*cf.* refs. 11, 12). This antibody reacts with some epithelial cells, such as colonic adenocarcinoma cell lines, but is unreactive with O erythrocytes. The absence or minimal expression on erythrocytes of type 1 blood group chains, in contrast to type 2 chains, has previously been noted by Hakomori and co-workers[10]. The failure of an anti-H type 1 antibody to agglutinate O erythrocytes is similar to the unreactivity of anti-A type 1 antibodies with A erythrocytes[13,14].

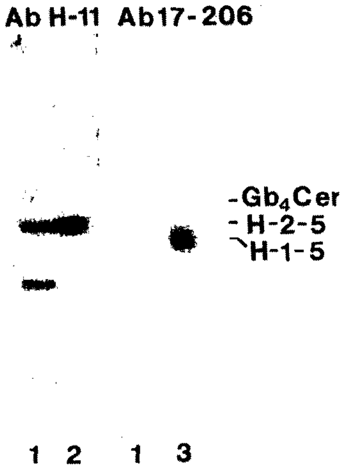

Fig. 2. Immunostaining by Ab 17-206 and AbH-11 of glycolipids separated by t.l.c.: (1) Neutral glycolipids from O erythrocytes, (2) H-2-5, and (3) H-1-5. See legend to Fig. 1B for abbreviations used. Antibody dilution: 1:500 of ascites fluid.

EXPERIMENTAL

Monoclonal antibody production and characterization. — A female NZB mouse (*cf.* ref. 5) was immunized six times intraperitoneally at 1–2-week intervals with suspensions (10^7 cells for each immunization) of the human colonic cancer cell line SW403. Splenocytes were fused with mouse SP 2/0 cells according to the procedure of Kohler and Milstein[1], as described by Dippold *et al.*[15]. Antibody-producing hybridomas were tested on the immunizing cell line, a melanoma cell line (SK-MEL-30), and a astrocytoma cell line (AJ) with a protein A red cell rossetting assay[15]. Clones that reacted with SW403 but not with the other two cell lines were retested on a panel of blood group-active glycoproteins (see below). A clone (17-206) reactive with a HLe^b glycoprotein was subcloned twice and grown, as an intraperitoneal tumor, in a mouse to produce antibody-containing ascites fluid. The subclass of the antibody was shown to be IgG3 by reactivity with sub-class specific antisera in an ELISA (Zymed Laboratories, San Francisco, CA). Anti-type 2 H antibody (H-11) has been described[9].

Glycoprotein and glycolipid analysis. — A panel of blood group-reactive glycoproteins isolated from ovarian cyst fluids having A, B, H, Le^a, Le^b, X (Le^x), Y (Le^y), and precursor specificities has been described[16]. Their reactivity with antibody was determined by an ELISA, which has also been described[14], except that protein A–alkaline phosphatase (Zymed Laboratories) at a dilution of 1:250 was used in the final step.

Glycolipids were isolated from human erythrocytes, meconium, and intestine, and from dog intestine as summarized by Lloyd *et al.*[6]. H-1-5, specifically, was derived from human meconium and characterized as described previously[17].

Reactivity with eight blood group-active glycolipids was determined by an ELISA as described[6], except that protein A–alkaline phosphatase, at a dilution of 1:250, was used instead of anti-mouse IgG antibody.

T.l.c. and immunostaining. — Glycolipids were separated by t.l.c. on aluminum-backed silica gel plates in 60:35:8 chloroform–methanol–water. Reactivity with antibody was detected by a modification of the method of Magnani *et al.*[7] as described[14]. Erythrocyte glycolipids were isolated as described by Furukawa *et al.*[14].

ACKNOWLEDGMENT

The authors thank Dr. Robert Knowles, Sloan-Kettering Institute, for providing antibody H-11.

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