Production of Monoclonal Antibodies Against IgG Allotypes and Their Use in Dot Immunobinding

Y. Tamaki and T. Kishida

Department of Forensic Medicine, Medical College of Oita, Oita 879-56, Japan

INTRODUCTION

IgG heavy chain allotypes, GM, are extremely useful in population genetics and in cases of disputed paternity. Unfortunately, the longstanding shortage of typing reagents hampers the widespread use of GM allotyping. Moreover, the cumbersome hemagglutination inhibition (HAI) method of typing is still generally used: O, D(+) red cells coated with GM-positive anti-D antibodies are agglutinated by the anti-GM serum; prior addition of a serum sample to the anti-GM serum inhibits the hemagglutination if the test serum contains that particular allotype. Despite its ingenuity, HAI poses several problems: (1) The shelf life of coated red cells is relatively short; thus the cumbersome step of coating must be repeated at frequent intervals. (2) Since human anti-GM sera are scarce and of low titer, a large number of healty individuals need continually to be screened for anti-GM antibodies so that the stock of antisera may not be exhausted. The screening is laborious and presupposes the availability of GM allotyping control sera and anti-D antibodies of various GM specificities which are extremely difficult to obtain from certain racial groups. (3) The occaisional occurrence in serum samples of antibodies to IgG and/or red cell antigens invalidates the HAI test unless they are absorbed by a time-consuming procedure. (4) The HAI test can not be used for the quantitative analysis of GM allotypes. All of these problems are solved by the use of monoclonal antibodies (MAbs) in enzyme-linked immunosorbent assay (ELISA) or dot immunobinding (DIB) (Fletcher et al. 1983; Kishida and Tamaki 1984; Bird et al. 1984; Francois-Gerard and Hoste 1987; Kishida et al. 1988; Weston-Kirkegaard et al. 1988; de Lange et al. 1989). Therefore, we produced the first MAb against the G3M G1 allotype, using a protein A-purified normal IgG3 protein as an immunizing antigen (Tamaki et al. 1984). Shortly thereafter, Bird et al. (1984) described MAbs to G1M F. Subsequently, de Lange et al. (1986, 1989) reported the production of MAbs to G1M Z, G1M A, G1M F, G3M B1/U, and G3M G1. Recently, Kimura et al. (1989) have prepared an anti-G3M T MAb. In producing anti-GM MAbs, the European workers immunized mice with myeloma proteins (which are not easily available) and/or used splenocytes as fusion partners after multiple boosts. In contrast, we immunized mice with a single dose of normal IgG suclass protein and used their lymph node cells as fusion partners to expedite MAb production. The present paper provides a practical approach to the production of anti-GM MAbs, and describes their usefulness in DIB in relation to their ELISA applications.

MATERIALS AND METHODS

The immunogens used for MAb production were IgG subclass ptroteins or their fragments prepared from normal human serum by DEAE-cellulose and protein A or Ricinus communis lectin I column chromatography as previously described

Advances in Forensic Haemogenetics 3
Edited by H. F. Polesky and W. R. Mayr

(Kishida 1985; Kimura et al. 1989): IgG3 (G3M G1), for anti-G3M G1; IgG3 (G3M B3ST), for anti-G3M T; $F(ab')_2$ fragments (practically G1M F) of IgG1-enriched IgG (GM AFB1B3), for anti-G1M F. BALB/c mice were immunized by a subcutaneous injection into hind feet of 20 ug antigen in FCA. On day 10, their popliteal lymph node cells were fused with P3U1 myeloma cells. The hybridomas were cultured, selected, screened for antibody production (by an inhibition-ELISA in IgG-coated plates), and cloned, by the standard technique except that autoclavable serum-free culture medium ASF-103 (Ajinomoto Co., Inc., Tokyo, Japan) was used. Antibody-producing hybridomas were grown in mice for collection of ascitic fluids.

For GM typing by DIB, serial double dilutions of serum samples in tris-buffered saline (TBS), pH 7.5, were applied in the form of a dot (0.5 ul), and detected with the MAb and HRP-labeled anti-mouse IgG. The incubation time was 30 min each; the diluent was 0.5% BSA in 0.02 M TBS/0.1% Tween 20; the color development reagent contained 30 mg 4-chloro-1-naphthol, 10 ml methanol, 50 ml TBS, and 25 ul of 30% hydrogen peroxide.

RESULTS AND DISCUSSION

Mouse IgG1 MAbs against G1M F, G3M T, and G3M G1 were produced whose titers were up to 1 : 640 000 in the indirect ELISA. None of the MAbs reacted with GM-negative IgG. DIB allowed detection only of the GM-positive sera out of 100 GM control sera previously typed by HAI. The lower limit of detection was dilutions from 1 : 256 to 1 : 1024 (Fig. 1).

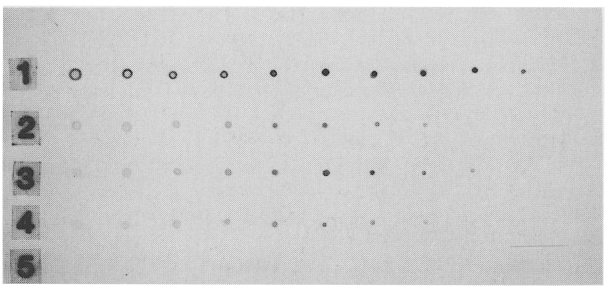

Fig. 1. G1M F typing by DIB of serial double dilutions (from left to right, 1/2 through 1/1024) of five serum samples of known GM phenotypes: 1, AFB1B3; 2, AG1FB1B3; 3, AXG1FB1B3; 4, AFB1B3; 5, AXG1.

Our purpose in producing anti-GM MAbs is not only to relieve the scarcity of human anti-GM sera but also to develop a new assay method to replace the cumbersome HAI method, thereby enabling the quantitation of allotype-specific IgG. To produce anti-GM MAbs, we persisted in using normal, and not myeloma, IgG subclass proteins as immunogens for their easy accessibility. The present study demonstrates that DIB is simple and practical in requiring only 2 h of bench work. In our experience, if more sensitivity is desired as in blodstain analysis, a direct-immobilization-ELISA is suitable in that serum dilutions of 1 : 10 000 to 1 : 40 000 can be readily GM-typed. A capture ELISA has an advantage over the above ELISAs in being far more sensitive. In fact, Francois-Gerard and Hoste (1987) and de Lange et al. (1989) detected GM allotypes in serum samples at dilutions of 1 : 320 000 to 1 : 2 000 000.

Regrettably, the capture ELISA has a counterbalancing disadvantage in being complex and expensive: plates need to be coated with purified anti-allotype or anti-IgG subclass MAbs as capture antibodies; therefore, it should be set aside for special purposes.

To the best of our knowledge, only eight anti-GM MAbs have been produced in the past six years. At this pace it may take ten more years to produce MAbs against the rest of the known GM allotypes. For our present purposes, however, it is sufficient to have available one MAb each against allotypes representing each haplotype. In the Japanese, for instance, the four haplotypes *GM*AZG1U, GM*AZXG1U, GM*AFNBOB1B3B4B5U* , and *GM*AZBOB3B5ST* with frequencies of 0.4369, 0.1725, 0.1297, and 0.2609, respectively (Matsumoto and Takatsuki, 1968), determine the nine GM phenotypes; accordingly, anti-G1M X, anti-G1M F (or anti-G3M B1), anti-G3M T, and anti-G3M G1 are essential requirements for routine phenotyping. We have already produced the latter three MAbs, but anti-G1M X is yet to be produced. Finally, the quantitative analysis of GM allotypes requires 100%-pure allotype-specific mollecules of IgG to be used as a standard in a competitive or capture ELISA. They can be isolated from IgG by affinity chromatography only on an anti-GM MAb column. For that purpose it is necessary to obtain large quantities of ascitic fluids containing anti-GM MAb.

REFERENCES

Bird P, Lowe J, Jefferis R, Lange GG de, Loghem E van, Ling N (1984) Monoclonal antibodies to an immunoglobulin allotype marker G1m(f). Vox Sang 47: 366-372

Fletcher SM, Dorrill MJ, Dolton P (1983) Bloodstain allotyping: an ELISA method for G1m(3). Rep 10th Int Congr Forens Haematogenet 221-226

Francois-Gerard Ch, Hoste B (1987) A double-sandwich ELISA test for G1m(3) phenotyping of bloodstains with a mouse monoclonal antibody. J Forens Sci Soc 27: 31-38

Kimura A, Tamaki Y, Kishida T, Fukuda M, Tsuji T (1989) Production of monoclonal antibody to human IgG allotype G3M T. J Immunol Methods 116: 99-103

Kishida T, Tamaki Y (1984) Gm typing by enzyme-linked immunosorbent assay (ELISA). Z Rechtsmed 93: 23-27

Kishida T, Tamaki Y, Fukuda M (1988) G3m(21) typing by ELISA and dot immunobinding with enzyme-labeled monoclonal anti-G3m(21) antibody. Z Rechtsmed 101: 15-20

Lange GG de, Leeuwen FM van, Eede PH van, Lincoln PJ, Engelfriet CP (1986) Monoclonal antibodies to immunoglobulin allotypes: specificity and reactivity in haemagglutination and ELISA techniques. Adv Forens Haemogenet 1: 157-161

Lange G.G de, Leeuwen AM van, Vlug A, Eede PH van, Engelfriet CP, Lincoln PJ (1989) Monoclonal antibodies against IgG allotypes G1m(z), G1m(a), G1m(f), G3m(b1/u) and G3m(g1): their usefulness in HAI and capture ELISA. Expl Clin Immunogenet 6: 18-30

Matsumoto H, Takatsuki K (1968) Studies on the Gm factors of Japanese population and families. Jpn J Legal Med 22: 635-642

Tamaki Y, Takahashi N, Kishida T, Ishikawa K (1984) Production and characterization of monoclonal anti-G3m(g) antibody. Jpn J Legal Med 38: 152-154

Weston-kirkegaard S, Southrada JM, Copouls B, Dykes DD, Polesky HF (1988) G1m(f) immunoblot procedure. Adv Forens Haemogenet 2: 466-469