Simplified Procedure for Preparation of Specific Antibodies to Gamma Globulins

RICHARD G. OLSEN, JAMES R. MCCAMMON, AND DAVID S. YOHN

Department of Veterinary Pathology, The Ohio State University, Columbus, Ohio 43210

Received for publication 2 April 1970

Antibodies were prepared to rhesus monkey, rabbit, dog, and hamster gamma globulins which had been purified by agar-block electrophoresis. Immunoelectrophoretic analysis demonstrated that these antisera were specific for gamma globulin components in whole serum. The use of these antisera as secondary serological reagents was examined.

Specific antibody to gamma globulin is used as the secondary reagent in the indirect immunofluorescence (10) and indirect radioiodine labeling antibody techniques (7). It is also used in the mixed cell hemagglutination (2) and antiglobulin consumption tests (4). The first two techniques may suffer from serological nonspecificity if the secondary reagent contains antibodies that react with other plasma proteins as well as the gamma globulins of the primary reagent. Ion-exchange chromatography has been extensively used (1, 6) to purify gamma globulin for immunization. Kurt et al. (5) were able to produce highly specific rabbit antirat gamma globulin antibody by using rat antibody-antigen complexes as a source of immunogen. This report describes a simple, reliable procedure for the preparation of specific antibodies to gamma globulin from several animal species by use of gamma globulin purified by agar-block electrophoresis.

MATERIALS AND METHODS

Gamma globulins from rabbit, rhesus monkey, dog, and hamster were partially purified by precipitation with an equal volume of 4 M (NH₄)₂SO₄ and washed twice with 2 M (NH₄)₂SO₄. The precipitates were re-suspended in distilled H₂O (0.5 original volume) and dialyzed against Veronal buffer at pH 8.2 with an ionic strength of 0.05. Approximately 4 ml (40 mg/ml) of protein was suspended in an equal volume of melted 2% agar at 45°C and subsequently subjected to electrophoresis in a 2% agar block (260 by 80 by 10 mm) for 10 hr at 25°C and at 150 v. A nonelectrophoresis grade of agar (Difco) was chosen to produce a strong electroosmotic flow during electrophoresis (8). Under these conditions, gamma globulins migrated toward the cathode, beta globulins remained at the origin, and albumin and alpha globulins migrated to the anode.

After electrophoresis, the gamma globulin component in the agar block was identified by removing a longitudinal section of the agar and fixing it with a mixture of methanol, water, and acetic acid (v/v, 4.5:4.5:1). The fixed gamma globulin component and the corresponding native globulin were used as immunogens. Native gamma globulins were eluted from agar by freeze thawing followed by low-speed centrifugation. Fixed gamma globulins were washed with distilled water, frozen, thawed, and eluted.

Adult white New Zealand rabbits were immunized with purified native or fixed gamma globulin (10 mg/ml) from either rhesus monkey, hamster, cat, or dog, whereas rhesus monkeys were used to prepare antibodies to rabbit gamma globulin. All animals were injected once with a total volume of 1 ml (one part immunogen to one part complete Freund's adjuvant) distributed to 10 sites in the thigh and cervical regions and, with rabbits, to the foot pads. All animals were bled 3 weeks later.

Immunoelectrophoretic analysis was performed by the procedure of Grabar and Burtin (3) on an LKB apparatus with the same agar and buffer as were used in agar-block electrophoresis.

RESULTS

Immunoelectrophoresis of whole gamma globulin antisera against whole serum from the species source of gamma globulin immunogen yielded lines of precipitation only with serum proteins that migrated as gamma globulin (Fig. 1). Essentially the same immunoelectrophoresis patterns were obtained with antisera prepared to fixed gamma globulins and with those prepared to native gamma globulins. Rabbit antimonkey gamma globulin, rabbit antihamster gamma globulin, and monkey antirabbit gamma globulin sera (Fig. 1A, B, C, respectively) each produced a heavy line of precipitation that corresponded to an immunoglobulin G (IgG) type globulin. In all instances, other gamma globulin components were detected that appeared to be IgM and IgA globulins. Rabbit antidog gamma globulin serum (Fig. 1D) reacted rather weakly with the IgG component,
although three other less heterodispersed gamma globulin components with lower diffusion coefficients were precipitated.

Rabbit antibodies to monkey gamma globulins and monkey antibodies to rabbit gamma globulins have been used extensively in this laboratory as the secondary reagent in indirect immunofluorescent tests in Yaba poxvirus studies (10). In similar studies, rabbit antidog gamma globulin serum has been used as an indirect immunofluorescent secondary reagent to detect dog auto-

antibodies in canine distemper-infected dog brain (A. Koestner, J. F. Long, R. O. Jacoby, R. G. Olsen, J. A. Shadduck, Proc. Int. Congr. Neural Pathol., 6th, Paris, France, in press). Rabbit antihamster gamma globulin has been successfully employed as an indirect secondary reagent with the paired radioiodine-labeled antibody (Yohn, Evans, and McCammon, Proc. Int. Cancer Congr., 10th, Houston, Tex., 1970, in press) and as an indirect immunofluorescent reagent in conjunction with adenovirus 12 T-antigen studies (9). It was found that secondary reagents prepared in this manner required no adsorption with other serum proteins to achieve serological specificity.

**DISCUSSION**

The principal result of this study was the development of a method for producing specific antibody toward agar-block purified gamma globulin from several animal species. The advantages of using these antisera as secondary reagents are threefold. (i) Unlike antisera prepared to (NH₄)₂SO₄-precipitated proteins, these reagents contain no antibodies toward plasma proteins other than gamma globulin. (ii) Immunoelectrophoresis indicates that the secondary reagents react with all classes of gamma globulin. The latter is of particular value if the class of immunoglobin antibody in the primary reagent is unknown. (iii) When these reagents are properly labeled, no adsorption is necessary. Although the amount of gamma globulin obtained by agar-block electrophoresis is relatively small, as compared to that obtained by column chromatography and various salting-out techniques, sufficient gamma globulin is available from a single run to immunize as many as 10 rabbits.

**ACKNOWLEDGMENTS**

The authors acknowledge the laboratory assistance of Larry E. Mathes and the photographic services of D. Q. Davis and staff. This investigation was supported by Public Health Service Grant CA 11613-01 from the National Cancer Institute.

**LITERATURE CITED**

1.eurmal, D. E., and R. T. Bowser. 1970. Purification of rat gamma globulin and the production of a specific antirat gamma globulin serum. Proc. Soc. Exp. Biol. Med. 133: 43–48.

2. Fagraeus, A., and J. Esparmi. 1961. Use of a “mixed hemadsorption” method in virus-infected tissue cultures. Nature (London) 190:370–371.

3. Grabar, P., and P. Burpin. 1964. Immunoelectrophoretic analysis, p. 26. American Elsevier Publishing Co., Inc., New York, N.Y.

4. Holmes, E. C., and D. L. Morton. 1969. Detection of antibodies against the mammary tumor virus with the anti-globulin consumption test. J. Nat. Cancer Inst. 42:733–738.
5. Kurt, J. B., H. C. Morse, III, and K. F. Austen. 1968. Biologic properties of rat antibodies. J. Immunol. 10:650–657.
6. Reif, A. E. 1969. Batch preparation of rabbit IgG globulin with DEAE-cellulose. Immunochemistry 6:723–731.
7. Sparks, F. C., C. C. Ting, W. G. Hammond, and R. B. Herberman. 1969. An isotopic antiglobulin technique for measuring antibodies to cell-surface antigens. J. Immunol. 102:842–847.
8. Wieme, R. J. 1965. Agar gel electrophoresis, p. 109–112. American Elsevier Publishing Co., Inc., New York.
9. Yohn, D. S., C. A. Funk, and J. T. Grace, Jr. 1967. Sex-related resistance in hamsters to adenovirus-12 oncogenesis. II. Influence of virus dose. J. Virol. 1:1186–1192.
10. Yohn, D. S., F. Marmol, and R. G. Olsen. 1970. Growth kinetics of Yaba tumor poxvirus after in vitro adaptation to cercopithecus kidney cells. J. Virol. 5:205–211.