Vol. 129, No. 1, 1985 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 31, 1985 Pages 206-212

PLATELET-ASSOCIATED IgG IS A SPECIFIC PROTEIN

Manfred Steiner

Division of Hematology/Oncology
The Memorial Hospital, Pawtucket, and
Brown University, Providence, RI

Received April 15, 1985

SUMMARY: The immunoglobulin binding to normal human platelets (PaIgG) was isolated on cell columns in which platelets or their membranes were attached via concanavalin A to an inert support matrix. Normal human IgG isolated from pooled serum was applied to the cell columns. The absorbed material which was eluted at low pH with a buffer of high ionic strength was immunologically and biochemically pure IgG. When the nonadherent IgG of the first passage through the platelet cell column was reapplied a second time virtually no IgG was retained. Isoelectric focusing on urea SDS polyacrylamide gels revealed only 2 major bands with pIs of 8.2 and 8.4 whereas the precolumn IgG contained a wide range of molecular species with pIs ranging from < 6.0 to 9.0.

Platelets contain an unusually large amount of IgG on their surface whose function remains unclear. The binding of this immunoglobulin has been studied with respect to its kinetics, its specificity and the nature of its receptor on the platelet membrane (1). Evidence for Fc receptors of IgG has been presented (2). If platelets indeed possess such receptors, it still remains questionable whether they are the only binding sites for IgG or there exist other receptors that bind the immunoglobulin via its Fab portion. The latter would lead to the obvious conclusion that cell specific IgG molecules are present in the blood that recognize a cell type. Such immunoglobulin species have been described (3,4).

The purpose of this investigation was to search for platelet-specific IgG (PAIgG) and to document its individuality. Our studies clearly indicate that PAIgG exists as a distinct entity. In addition, there may also be binding of some non-specific IgG.

MATERIALS AND METHODS

Isolation of Platelet and Platelet Membranes

Blood was collected from normal volunteers into CPD-(citrate phosphate dextrose solution, USP) containing blood bags. Platelet rich plasma was

0006-291X/85 $1.50

prepared as previously described (5). After acidification with 15% ACD (acid citrate dextrose solution, USP), the platelets were isolated by centrifugation, washed twice with 0.05 M phosphate buffer, pH 6.8 containing 0.14 M NaCl (PBS) and were then incubated in PBS for 30 min at room temperature to desorb as much platelet bound IgG as possible. After centrifugation, platelets were resuspended in PBS, pH 7.2 at a concentration of 2-3 x 10^9 cells/ml.

Membranes were isolated from glycerol-lysed platelets (6), washed and concentrated to 3-5 x 10^9 cells/ml. Both intact platelets and membrane suspensions were used for the preparation of cell columns.

Cell Column Chromatography

This method represents a modification of that described by Sela et al. (7). Sephadex G-50 was treated with concanavalin A (Con A) for 30 min at room temperature at a ratio of 1 mg of the lectin to 1 mg of the swelled support. Platelet membrane suspensions or concentrates of intact platelets were then added and the mixtures allowed to stand overnight before being degassed and poured into columns. After washing the column for 30 min with 30% glutaraldehyde the columns were equilibrated with PBS, pH 7.2. Before passing IgG over the cell column a 1% solution of bovine serum albumin in PBS, pH 7.2 was passed through. This was followed by exhaustive washing with PBS until the optical absorbance of the column effluent measured at 275 nm was < 0.005.

Preparation of IgG

IgG was isolated from serum that had been passed over Affi-gel blue (Bio-Rad, Richmond CA) columns. The protein that was not absorbed consisted of IgG and transferrin. To remove the latter, the concentrated excluded elution content containing the 2 proteins was applied to a DEAE-cellulose column eluted with 0.005 M PO_4^{2-} buffer, pH 8.0. The IgG fraction was concentrated and tested by SDS-polyacrylamide gradient gel electrophoresis (8) and by immunoelectrophoresis (Fig. 1). Both methods revealed virtually pure IgG. The concentration was adjusted to between 5.5-6.0 mg/ml.

Preparation of Platelet Associated IgG

Approximately 300-350 mg of IgG/100 ml Sephadex were passed through the cell column at a flow rate of 25-27 ml/hr. After washing with 350 ml PBS, pH 7.2/100 ml Sephadex, elution was begun with 0.05 M glycine buffer, pH 3.0 containing 0.5 M NaCl at a flow rate of 40 ml/hr. Fractions of 6.5 ml were collected and optical absorbance at 275 nm measured. The fractions containing the eluted protein peak were combined and concentrated. The concentrated protein was then dialyzed against several changes of 0.05 M phosphate buffer, pH 7.0. The protein concentration of the final sample was measured by the Lowry method (9) using bovine serum albumin as standard. Washing of the prepared cell column with the elution buffer containing 0.5 M NaCl did not remove any protein. The cell columns could be reused several times. After each run the columns were exhaustively washed with PBS, followed by passage of 1% serum albumin in PBS and more PBS until the optical absorbance had returned to baseline values.

Isoelectric Focusing Gel Electrophoresis

Nonidet-urea polyacrylamide focusing gels were made according to O'Farrell (10) using a pH gradient from 5-10. The samples were not reduced prior to application to the gels. The gels were cast in glass cylinders measuring 125 x 5 mm. After focusing for 12 hours at 400 V and 1 hour at 800 V, the gels were extruded, fixed by immersion in 30% methanol/10% trichloracetic acid/3.5% sulfosalicylic acid for 1 hour and in 30% methanol/12% trichloracetic acid for 3 hours or longer. After that the staining

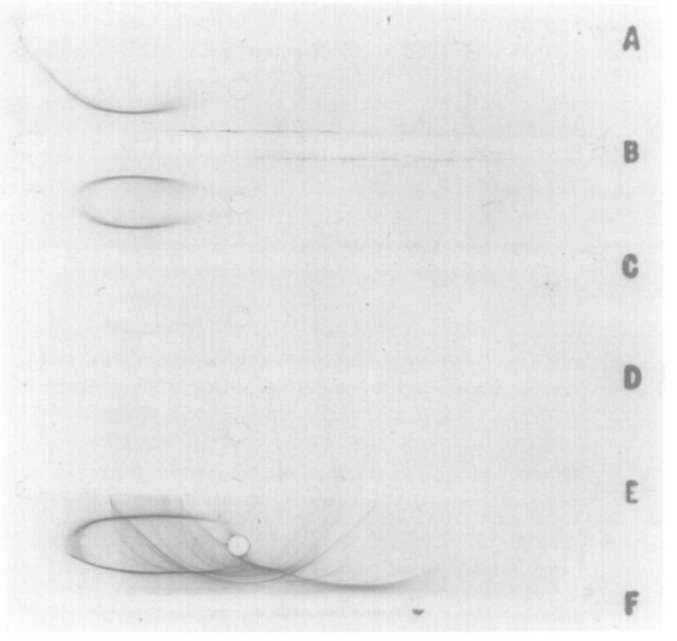


Figure 1. Immunoelectrophoresis of pooled human serum IgG and material eluted from a platelet column. After electrophoretic separation, each sample was reacted overnight with rabbit antibody against whole human serum or human IgG. The plate was stained with Coomassie blue. Precolumn IgG, row B (antibody against human IgG) and C (antibody against human serum); IgG eluted from the cell column, row D (antibody against human serum) and E (antibody against human IgG); normal human serum (control) row F (antibody against human serum).

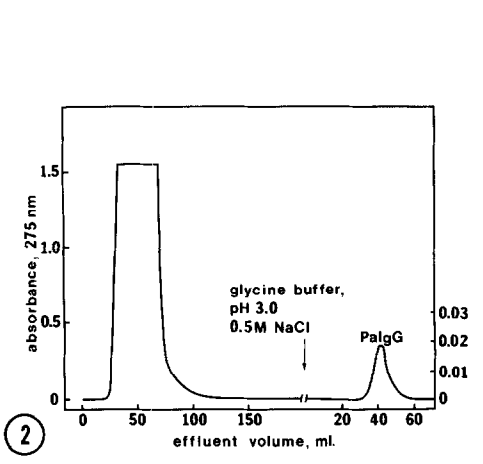
protocol described by BioRad (11) was followed utilizing the commercially available staining kit (Bio-Rad; Richmond, CA).

Steric Exclusion Chromatography

Molecular size determinations and analysis of cell column effluents was accomplished by HPLC on a μ-Spherogel column TSK 3000 SW (Beckman; Berkley, CA) measuring 7.5 x 600 mm. The solvent used was 0.01 M sodium phosphate buffer, pH 6.5 containing 0.15 M NaCl. The column was operated at ambient temperature at a flow rate of 0.8 ml/min. The optical absorbance of the column effluent was monitored at 214 and 275 nm.

RESULTS

When pure human IgG was passed over a cell column containing either intact platelets or platelet membranes, the overwhelming majority of the applied protein did not adhere (Fig. 2). After the optical absorbance had returned to baseline value, at least 100 ml of additional equilibration buffer was passed through the column before the retained protein was eluted. A small peak of material with optical absorbance at 275 nm was removed by



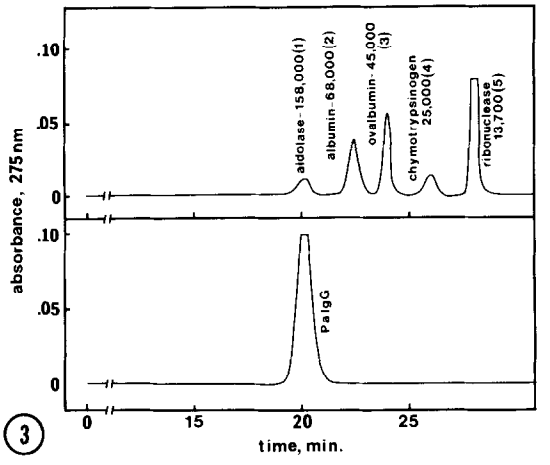
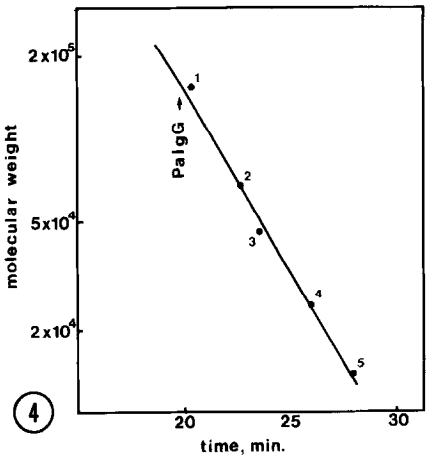


Figure 2. Elution of PaIgG from a platelet column. Pooled human serum IgG was applied to the column. After washing exhaustively with PBS, pH 7.2, elution with 0.05 M glycine buffer, pH 3.0 containing 0.5 NaCl was begun.

Figure 3. Steric exclusion chromatography of a series of standard proteins (upper panel) and of material eluted from the cell column (lower panel) on a μ-Spherogel TSK 3000 SW column.

the low pH and high ionic strength elution buffer. After concentration, this peak was evaluated by immunoelectrophoresis (Fig. 1). A pure preparation of IgG was apparent which could be confirmed by SDS-polyacrylamide gel electrophoresis. Under complete reduction and alkylation the material showed 2 major bands corresponding to the heavy and light chains of the immunoglobulin.

The protein that was eluted from the cell column was analyzed by gel filtration chromatography using an HPLC system. The column used for these studies (μ-Spherogel TSK 3000 SW) had an exclusion limit of 3×10^5 for proteins. Calibration of the column with a set of standard proteins of molecular weights ranging between 158,000 and 13,700 allowed determination of the apparent molecular weight of the material eluted from the cell column (Fig. 3). As shown in figure 4, the peak corresponded to a protein of M.W. 150,000. Complete reduction with subsequent alkylation gave evidence of 2 components, one of M.W. 50,000 and the other of M.W. 24,000 (Fig. 5).



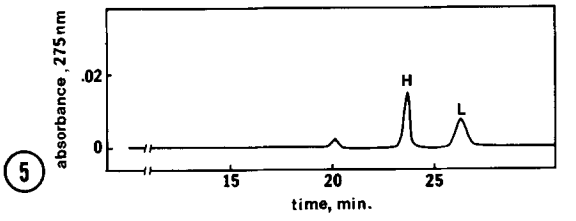


Figure 4. Molecular weight calibration curve of proteins shown in figure 3.

Figure 5. Steric exclusion chromatography on a μ-Spherogel TSK 3000 SW column of completely reduced and alkylated PaIgG eluted from the platelet column. H: heavy chain, L: light chain.

The IgG that did not adhere to the cell column was concentrated and reapplied to the cell column. From this 2nd application no platelet specific IgG was retained on the cell column.

The protein eluted from the platelet column was also analyzed by isoelectric focusing electrophoresis. Normal IgG extracted from human plasma showed a large number of bands ranging in pI from 6.0 to 9.0 (Fig. 6). The IgG retained on the columns showed only 2 bands. These had apparent pIs of 8.2 and 8.4.

DISCUSSION

These studies strongly indicate that the IgG that normally binds to platelets is not a random species of IgG but rather a very specific molecular moiety. Although a number of bands could be visualized by isoelectric

Figure 6. Isoelectric focusing of normal pooled human IgG (A) and PaIgG eluted from the cell column (B) on urea-SDS polyacrylamide gels.

focusing, most were extremely faint compared to the 2 major ones. Serum IgG normally has a wide range distribution of isoelectric points which cover a pH range from below 6.0 to pH 9.0. The identity of the material isolated from the cell column containing immobilized platelets or platelet membranes fulfilled all the criteria of an IgG molecule. Detailed studies furthermore showed that it represented the only protein that was eluted from the cell column. The fact that normal serum IgG that was absorbed once failed to adhere to the cell column is confirmatory evidence for the specificity of the platelet associated IgG. This investigation does not refute the hypothesis that platelets contain Fc receptors as the cell-specific IgG that was identified could reflect the presence of an immunoglobulin of a particular Fc group.

The existence of natural antibodies has been reported in various animal species (3,12-19). These were often found to be directed against various autoantigens, thus not clearly adhering to the definition provided by Boyden (20). Such antibodies may arise for a variety of reasons. Cell senescence may be one of them. The emergence of altered membrane glycoproteins during red cell aging has been described (4,5). As IgG may play a role in the removal of senescent erythrocytes (4,21,22), platelets and other cells, one could speculate that immunoglobulins exist that have specificity for such "altered" surface proteins of cells. The specific platelet associated IgG found in these studies may be a representative of such a group of antibodies that are directed against senescence antigens. In vivo aging of platelets is accompanied by increasing amounts of PAIgG (23). The verification of this hypothesis will have to wait for the eventual discovery of the specific antigen(s) for this antibody.

ACKNOWLEDGEMENTS

This study was supported by research grant HL 25698 of the National Heart, Lung and Blood Institute. The expert assistance of Ms. Saundra Patrick is gratefully acknowledged.

REFERENCES

1. Sugiura, K., Steiner, M. and Baldini, M. (1980) Biochim. Biophys. Acta. 631, 166-176.
2. Cheng, C.M. and Hawiger, J. (1979) J. Biol. Chem. 254, 2165-2167.
3. Sela, B.A., Wang, J.L. and Edelman, G.M. (1975) Proc. Natl. Acad. Sci. USA 72, 1127-1131.
4. Low, P.S., Waugh, S.M., Zinke, K., and Drenckhahn, D. (1985) Science 227, 531-533.
5. Ando, Y., Steiner, M., and Baldini, M. (1974) Transfusion 14, 453-461.
6. Ando, Y.and Steiner, M. (1973) Biochim. Biophys. Acta 311, 26-37.
7. Sela, B.A. and Edelman, G.M. (1977) J. Exptl. Med. 145, 443-449.
8. Clemetson, K.J., Capitanio, A. and Lüscher, E.F. (1979) Biochim. Biophys. Acta. 553, 11-24.
9. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
10. O'Farrell, P.H. (1975) J. Biol. Chem. 250, 4007-4021.
11. Bio-Rad Bulletin 1089 (1982) Bio-Rad Laboratories, Richmond, CA.
12. Cunningham, A.J. (1974) Nature 252, 749-751.
13. Martin, S.W., and Martin, W.J. (1975) Proc. Natl. Acad. Sci. 72, 1036-1040.
14. Martin, W.J. and Martin, S.E. (1975) Nature 254, 716-718.
15. Meredith, P.J., Kristie, J.A. and Walford, R.L. (1979) J. Immunol. 123, 87-91.
16. Shinomiya, T., and Koyama, J. (1974) Immunochemistry 11, 467-473.
17. Strejan, G.H., Smith, P.M., Grant, C.W. and Surlan, D. (1979) J. Immunol. 123, 370-378.
18. Karsenti, E., Guilbert, B., Bornens, M., and Avrameas, S. (1977) Proc. Natl. Acad. Sci. 74, 3997-4001.
19. Guilbert, B., Dighiero, G. and Avrameas, S. (1982) J. Immunol. 128, 2779-2787.
20. Boyden, S.V. (1965) Adv. Immunol. 5, 1-28.
21. Kay, M.M.B. (1981) Nature 289, 491-494.
22. Kay, M.M.B. (1975) Proc. Natl. Acad. Sci. USA 72, 3521-3535.
23. Sugiura, K., Steiner, M. and Baldini, M. (1981) Thromb. Haemostas. 45, 27-33.