IDENTIFICATION OF THE MEMBRANE GLYCOPROTEIN THAT IS THE C3b RECEPTOR OF THE HUMAN ERYTHROCYTE, POLYMORPHONUCLEAR LEUKOCYTE, B LYMPHOCYTE, AND MONOCYTE*

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The plasma membranes of human erythrocytes (E)[1] (1), polymorphonuclear leukocytes (PMN) (2, 3), monocytes (4), macrophages (2), B lymphocytes (2), mast cells (5), and visceral epithelial cells of the glomerulus (6) have receptors for C3b, the major cleavage fragment of C3, that mediate the attachment to these cells of complexes that bear this complement protein. Studies of the function of the C3b receptor on these cells have defined its participation in phagocytic (3, 7–12) and secretory reactions (13–15), but physiocochemical characterization of the receptor is limited to the demonstration of its trypsin sensitivity (2, 4), which suggests that it is a protein.

Recently, a 205,000-mol wt glycoprotein (gp205) with inhibitory activity in the human alternative complement pathway was solubilized from human E membranes with Nonidet P-40 (NP-40) and was purified to homogeneity by use of chromatographic procedures that included reversible adsorption to Sepharose-C3 (16). Purified gp205 was shown to accelerate the decay of the properdin (P)-stabilized amplification C3 convertase (C3b,Bb,P) by displacing Bb from C3b, and to promote cleavage of the α-polypeptide chain of C3b by C3b inactivator (C3bINA). As these inhibitory functions of gp205, and its capacity to bind to Sepharose-C3, indicated an affinity of this membrane-derived glycoprotein for C3b, its possible identity as the C3b receptor was examined.

This study demonstrates that rabbit antibody to gp205 inhibits C3b receptor function not only on human E, but also on PMN, B lymphocytes, and monocytes. Interaction of anti-gp205 with solubilized, radioiodinated membrane proteins from each of these cells immunoprecipitates a single membrane protein with an apparent 205,000 mol wt on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Thus, the C3b receptor of these four cell types is gp205.

[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; C3b,Bb,P, properdin-stabilized amplification C3 convertase; C3bINA, C3b inactivator; DFP, diisopropyl fluorophosphate; E, erythrocyte; EA, erythrocytes sensitized with rabbit IgG; FCS, fetal calf serum; gp205, a 205,000 mol wt glycoprotein; NP-40, Nonidet P-40; P, properdin; PBS, phosphate-buffered saline; PBS-BSA-SBTI, PBS containing 2 mg BSA/ml and 1 mg soybean trypsin inhibitor/ml; PMN, polymorphonuclear leukocyte; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Materials and Methods

Preparation and Assay of Rabbit Antibody to gp205. gp205 was solubilized from membranes of human E by use of NP-40 (Shell Chemical Co., New York) and was purified by sequential cation-exchange chromatography, affinity chromatography on Sepharose-C3, gel filtration, and affinity chromatography on Sepharose- (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J.) lentil lectin (Sigma Chemical Co., St. Louis, Mo.) (16). Purified gp205 was homogeneous when analyzed by SDS-PAGE, with or without prior reduction of disulfides. A rabbit was immunized with 75 μg of gp205 emulsified in Freund's complete adjuvant and boosted 5 wk later by intramuscular injection of an additional 50 μg in incomplete Freund's adjuvant. Antiserum was obtained from the rabbit 2 wk after boosting, and the IgG fraction was prepared by precipitation in 50% ammonium sulfate and chromatography on DE52-cellulose. The IgG was digested (17) with pepsin (Worthington Biochemical Corp., Freehold, N. J.) and the F(ab')$_2$ fragments were isolated by gel filtration of the digest on Sephadex G-200 (Pharmacia Fine Chemicals, Div. of Pharmacia, Inc.). Serum that had been obtained from the same rabbit before immunization was processed in an identical manner to prepare preimmunization IgG and F(ab')$_2$. Both preimmunization F(ab')$_2$ and anti-gp205 F(ab')$_2$ demonstrated single protein bands having apparent 100,000 mol wt on SDS-PAGE. Anti-gp205 F(ab')$_2$ was labeled to a specific activity of 100,000 cpm/μg with ^{125}I (New England Nuclear, Boston, Mass.) by use of insoluble lactoperoxidase (Worthington Biochemical Corp.) (18).

Neutralization by anti-gp205 IgG of the capacity of purified gp205 to accelerate decay of C3b,Bb,P was measured by hemolytic assay. Dilutions of preimmunization IgG or anti-gp205 IgG were incubated for 60 min at 4°C with 2 ng gp205 in 0.1 ml of veronal-buffered saline that contained 0.1% gelatin and 20 mM EDTA. 1 × 10^7 sheep E that bore 1.5 C3b,Bb,P sites/cell (19) in 0.1 ml of this buffer were added, and incubation was continued for 20 min at 30°C. The residual C3b,Bb,P sites were hemolytically developed by addition of 0.3 ml of rat serum diluted in veronal-buffered saline containing 40 mM EDTA, and incubation for was continued 60 min more at 37°C. The percent hemolysis was measured and the average number of hemolytic sites/cell was calculated.

Assay of Complement Receptors on Peripheral Blood Cells by Formation of Rosettes with Sheep E That Bore C3b, C3bi, or C3d. C3 (20), B (21), D (19), P (22), β1H (23), and C3bINA (24) were purified to homogeneity as assessed by SDS-PAGE. C3 was radiolabeled with ^{125}I to a 20,000 cpm/μg sp act by use of insoluble lactoperoxidase (18). Sheep EC3b that bore 8,500 ^{125}I-C3b/E were prepared by interaction of trypsinized E with ^{125}I-C3, B, and D, as described (25). Sheep EC3bi were prepared by incubation of the EC3b with 5 μg C3bINA/ml and 50 μg β1H/ml for 45 min at 37°C. This treatment abolished the capacity of these cells to form cell-bound C3b,Bb,P sites, but did not decrease their content of cell-bound ^{125}I (26). Sheep EC3d were prepared by incubating the EC3bi with 0.1 μg tryspin/ml for 40 min at 37°C, which resulted in a 90% decrease in cell-bound ^{125}I (26).

Human E were obtained from fresh, citrated blood and were washed five times in phosphate-buffered saline (PBS) that contained 20 mM NaPO$_4$, 120 mM NaCl, pH 7.3, and the buffy coat and the top 10% of E were removed after each centrifugation. PMN were isolated from citrated blood by dextran sedimentation of E, and centrifugation of the leukocytes on Ficoll-paque cushions (Pharmacia Fine Chemicals, Div. of Pharmacia Fine Chemicals) (27). Residual contaminating E were hypotonically lysed in 0.2% NaCl and the PMN were washed free of hemoglobin. The final preparation consisted of 98% viable PMN by morphology and exclusion of trypan blue. A population of cells enriched for B lymphocytes was obtained from peripheral blood mononuclear cells by sequential removal of cells forming rosettes with neuraminidase-treated sheep E (28, 29) and of cells adherent to plastic Petri dishes (30). The final preparation was 96% viable by exclusion of trypan blue and contained 56% cells with membrane Ig, as assessed by immunofluorescence with fluorescein-conjugated rabbit F(ab')$_2$ anti-human F(ab')$_2$ (N. L. Cappel Laboratories Inc., Cochranville, Pa.), and 2% cells capable of ingesting sheep E sensitized with rabbit IgG (EA). Monocytes were isolated directly from the mononuclear cells by adherence to glass coverslips and were 86–91% phagocytic, as assessed by ingestion of sheep EA.

1 × 10^6 E, PMN, and B lymphocytes, respectively, were incubated for 30 min at 30°C with 1 × 10^7 sheep E that bore C3b, C3bi, or C3d in 0.2ml PBS that contained 2 mg bovine serum

albumin/ml and 1 mg soybean trypsin inhibitor/ml (PBS-BSA-SBTI). These reaction mixtures also contained variable concentrations of preimmunization and anti-gp205 F(ab')$_2$, respectively, ranging from none to 12.8 μg/ml. Monolayers of monocytes were overlaid with 0.2 ml of this buffer that contained 1 × 10^7 of the sheep E intermediates and the same concentrations of the preimmunization and anti-gp205 F(ab')$_2$ and were incubated for 30 min at 37°C. To assess the formation of rosettes in the cell suspensions, a drop was placed on a microscope slide and covered with a coverslip; 200 human cells were then examined at × 900 magnification. Formation of rosettes by monocytes was also microscopically assessed after rinsed coverslips carrying the monocytes were placed over drops of PBS-BSA-SBTI on microscope slides. Binding of the two or more sheep E to an human E, and three or more sheep E to a PMN, B lymphocyte, or monocyte was considered a rosette.

Assay of Binding of ^{125}*I-F(ab')$_2$ Anti-gp205 to Human Peripheral Blood Cells.* 1.2 × 10^9 human E, 3.3 × 10^7 PMN, 5.9 × 10^7 peripheral blood mononuclear cells enriched for B lymphocytes and that contained 52% membrane-Ig-bearing cells and 60% cells capable of forming rosettes with sheep EC3b, and 7 × 10^7 cells of a preparation of T lymphocytes that had been isolated by rosette formation with neuraminidase-treated sheep E (28, 29) were suspended at 4°C in 1 ml of PBS-BSA-SBTI. To five replicate samples of 75 μl of each cell type were added 100 μg preimmunization F(ab')$_2$ in 20 μl PBS, and 100 μg F(ab')$_2$ anti-gp205 was added in PBS to an additional five replicate samples. After incubation for 20 min at 4°C, pairs of samples containing the pre- and postimmunization F(ab')$_2$ received 10 μl of PBS that contained increasing amounts of ^{125}I-F(ab')$_2$ anti-gp205, ranging from 0.08 μg to 2.5 μg, and incubation was continued for 60 min at 4°C while the cells were intermittently agitated. Duplicate 40 μl samples of each reaction mixture were layered on 300 μl of a mixture of six volumes of dibutyl phthalate and four volumes of dinonyl phthalate in 400 μl microtest tubes. After centrifugation at 8,000 g for 75 sec in a Beckman B microcentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.), tubes were cut to yield tips containing the cell pellets and upper portions containing the cell-free aqueous phases. Each was assessed for ^{125}I to measure cell-bound and unbound ^{125}I-F(ab')$_2$, respectively.

To assay binding of ^{125}I-F(ab')$_2$ anti-gp205 to monocytes, 4 × 10^6 mononuclear cells from the Ficollpaque cushions in 0.2 ml RPMI 1640 medium (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 10% heat-inactivated fetal calf serum (FCS) were added to each of 22 round-bottomed 1- × 10-cm glass tubes, and incubated for 30 min at 37°C in the presence of 5% CO$_2$. Nonadherent cells were removed from the tubes by the addition of 3 ml PBS-BSA-SBTI, light agitation of the tubes, removal of the supernates, and repetition of this procedure. The washed monolayers of monocytes in 10 tubes received 200 μg of preimmunization F(ab')$_2$ in 140 μl PBS-BSA-SBTI and an additional 10 monolayers received 200 μg of unlabeled F(ab')$_2$ anti-gp205. After incubation for 20 min at 4°C, pairs of tubes containing preimmunization and anti-gp205 F(ab')$_2$, respectively, received 10 μl PBS-BSA-SBTI that contained increasing amounts of ^{125}I-F(ab')$_2$ anti-gp205 ranging from 0.31 to 5 μg, and incubation was continued for 60 min at 4°C. All tubes received 1 ml of ice-cold PBS-BSA-SBTI and were centrifuged at 900 g for 10 min at 4°C; the supernates were removed and assessed for unbound ^{125}I-F(ab')$_2$. The cells were washed twice by repetitive additions of 1 ml of this buffer, centrifugation, and aspiration of the supernates. Cell-associated ^{125}I-F(ab')$_2$ was measured in the material released from the washed monolayers by addition of 1 ml PBS-BSA-SBTI that contained 0.5% NP-40. The number of cells in the monolayers was estimated as 1 × 10^6 by counting the nuclei released after detergent solubilization of cells in two additional tubes containing monolayers (31).

Surface Radioiodination of Peripheral Blood Cells and Identification of the Membrane Proteins Reacting with Anti-gp205. Human E, 5 × 10^8 cells, were suspended in 15 ml of PBS at 0°C with constant stirring, and 500 μg lactoperoxidase (Sigma Chemical Co., St. Louis, Mo.), 750 μCi of Na[^{125}I], and 0.015 μmol H$_2$O$_2$ were sequentially added (32, 33). During continued incubation at 0°C, addition of H$_2$O$_2$ was repeated at 3, 6, and 9 min, and addition of lactoperoxidase was repeated at 6 min. After 12 min, the E were sedimented by centrifugation at 4°C, washed once with ice-cold PBS, and washed three times with PBS-bovine serum albumin (BSA). The E were lysed by suspension in 15 ml of buffer that contained 5 mM NaPO$_4$, 0.5 mM EDTA, pH 8.0, at 4°C. The membranes were pelleted by centrifugation at 48,200 g for 40 min at 4°C, and

were washed by resuspension in 15 ml of this buffer and recentrifugation. In four separate experiments, 96–98% of ^{125}I that was associated with the labeled intact E was recovered in the washed membranes. The membranes were suspended in 2 ml of PBS that contained 0.5% NP-40 and 5 mM diisopropyl fluorophosphate (DFP) (Sigma Chemical Co.), held at 0°C for 60 min, and centrifuged at 48,200 g for 40 min. The supernates, which contained the solubilized membrane proteins, were removed and stored at −70°C.

1×10^8 PMN were iodinated with 750 μCi of ^{125}I by the procedure described for labeling of E, except that the volume and reagents utilized in the reaction were reduced fivefold. In two separate experiments, 92 and 94% of the PMN excluded trypan blue after iodination. The PMN were suspended by vigorous vortexing in 2 ml of ice-cold PBS that contained 0.5% NP-40 and 5 mM DFP, held at 0°C for 60 min and centrifuged at 48,200 g for 60 min. The supernates that contained solubilized membrane proteins were removed and stored at −70°C.

Mononuclear cells, 2.5×10^8, which had been depleted of cells that formed rosettes with neuraminidase-treated sheep E, were iodinated with 1 mCi of ^{125}I by the procedure described for E with a twofold reduction in the reaction volume and reagents. 2.5×10^8 labeled mononuclear cells in 5 ml of RPMI-1640 medium that contained FCS were added to each of five plastic Petri dishes of 100-mm diameter and were incubated for 30 min at 37°C in the presence of 5% CO_2. The Petri dishes were gently agitated and the supernates that contained 7.2×10^7 nonadherent cells were removed. The monolayers were washed by twice adding and removing 5 ml of PBS containing 2 mg BSA/ml. The nonadherent cells were washed three times with this buffer by repeated suspension and centrifugation. In two experiments, the nonadherent cells were 91 and 93% viable by trypan blue exclusion; 2 and 4% of the cells were capable of ingesting sheep EA, 56 and 65% were capable of forming rosettes with sheep EC3b, and 52 and 63% reacted with fluorescein-conjugated rabbit F(ab')₂ anti-human F(ab')₂. In two experiments, the adherent cells were 91 and 94% viable; 82 and 89% of the cells were capable of ingesting sheep EA. The adherent cells were removed from the Petri dishes by the addition of 5 ml PBS that contained 0.5% NP-40 and 5 mM DFP to one dish, swirling, and passing the buffer to the next dish. The released material was held at 0°C for 60 min and then centrifuged at 48,200 g for 40 min; the supernate was removed and stored at −70°C. Membrane proteins of nonadherent cells were solubilized and obtained by the procedure described for E and PMN.

The solubilized membrane proteins from each cell type were partially purified by affinity chromatography on Sepharose-C3 (16). The supernates of the NP-40 extracts were diluted with six volumes of 10 mM $NaPO_4$, pH 7.1, that contained 2 mg BSA/ml and 5 mM DFP, and were applied to 1.5-ml columns of Sepharose-C3. The columns were washed with the diluting buffer and were eluted by application of this buffer that contained 400 mM NaCl and 0.5% NP-40.

Solubilized ^{125}I-labeled membrane proteins in 2 ml of the buffer utilized for elution of Sepharose-C3 were immunoprecipitated by the addition of 50 μg of preimmunization IgG or anti-gp205 rabbit IgG, incubation for 60 min at 4°C, and addition of 100 μl of a 10% suspension of *Staphylococcus aureus*, Cowan I strain (34). After incubation for 30 min at 4°C, the bacteria were sedimented by centrifugation at 5,000 g for 15 min and washed 3 times. Proteins bound to the bacteria were released by suspending the particles in 200 μl of PBS that contained 2% SDS and 5% 2-mercaptoethanol at 100°C for 1 min. The released material was subjected to SDS-PAGE according to the method of Laemmli (35) and ^{125}I was assayed in 1-mm sections of the gels.

Results

Rabbit Antibody to gp205 of Human Erythrocytes Inhibits C3b-Receptor Function of Peripheral Blood Cells. The IgG fractions of sera that had been obtained before and after immunization of a rabbit with purified gp205 of human E were compared for the capacity to neutralize the inhibitory activity of gp205. Incubation of sheep E that bore ∼1.5 C3b,Bb,P sites/cell with buffer alone for 20 min at 30°C resulted in 0.93 residual sites/cell, wheres incubation of the cells with 10 ng gp205/ml decreased the number of C3b,Bb,P sites/cell to 0.16. Preimmunization rabbit IgG did not impair the inhibitory activity of gp205. In contrast, postimmunization IgG neutralized gp205

in a dose-response manner, and complete blocking of inhibition was achieved with concentrations of 2 μg IgG/ml or higher (Fig. 1), indicating that the postimmunization rabbit IgG had specificity for gp205.

The capacity of anti-gp205 to impair C3b-receptor function was assessed. Human E, PMN, monocytes, and B lymphocytes were incubated with sheep EC3b in buffer alone or buffer that contained increasing concentrations of F(ab')₂ anti-gp205 and the percent of human cells forming rosettes was quantitated. Anti-gp205 inhibited in a dose-response manner the capacity of each type of cell to bind sheep EC3b (Fig. 2), with 50% inhibition occurring at 0.13 μg/ml, 0.90 μg/ml, 1.20 μg/ml, and 1.25 μg/ml of F(ab')₂ for E, B lymphocytes, PMN, and monocytes, respectively. 1 mg/ml of preimmunization F(ab')₂ did not impair rosette formation by any of the four types of cells. Thus, rabbit antibody that neutralizes the inhibitory activity of gp205 also impairs the function of C3b receptors of peripheral blood cells.

The monospecificity of anti-gp205 for the C3b receptor was analyzed by examining the effect of 20 μg F(ab')₂/ml on the formation of rosettes by monocytes and B lymphocytes with sheep EC3b, EC3bi, and EC3d. Almost all monocytes bound sheep EC3b, approximately one-half that number bound EC3bi, and none had adherent EC3d (Table I). The frequency of rosettes in the B lymphocyte population also was greater with EC3b than with EC3bi, and a small proportion bound EC3d. The single high concentration of F(ab')₂ anti-gp205 that totally inhibited C3b receptor-dependent rosette formation by both types of cells had no effect on the function of their C3bi and C3d receptors.
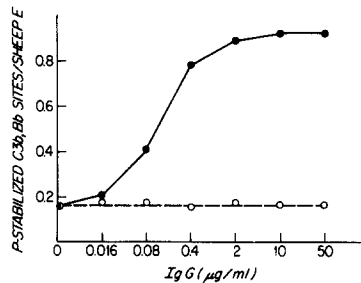

Fig. 1.   Dose-response relationship between the concentration of preimmunization (O) and postimmunization (●) rabbit-IgG with which gp205 had been preincubated, and the capacity of gp205 to inhibit C3b,Bb,P sites on sheep E during subsequent incubation for 20 min at 30°C. The number of C3b,Bb,P sites/sheep E that had been incubated with buffer instead of gp205 was 0.93.
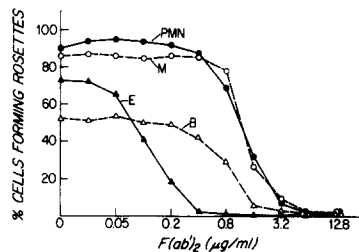

Fig. 2.   Dose-response relationship between the concentration of F(ab')₂ anti-gp205 and the percent of human E (▲), B lymphocytes (△), monocytes (O), and PMN (●) forming rosettes with sheep EC3b.

TABLE I

Percent of Cells in Preparations of Monocytes and B Lymphocytes that Form
Rosettes with Sheep Erythrocytes that Bear C3b, C3bi, or C3d in the Presence
or Absence of F(ab')₂ anti-gp205

Cell preparation	EC3b	EC3bi	EC3d
		%	
Monocyte	82	36	0
Monocyte + anti-gp205	0	38	0
B lymphocyte	64	12	4
B lymphocyte + anti-gp205	0	14	4

To determine whether the differing doses of F(ab')₂ anti-gp205 that were required for 50% inhibition of C3b receptor function of human E, B lymphocytes, PMN, and monocytes reflected variable numbers of antigenic sites on these cells, the uptake of ^{125}I-F(ab')₂ anti-gp205 by each of the four types of cells was quantitated. Binding of ^{125}I-F(ab')₂ anti-gp205 to each cell type in the presence of excess unlabeled F(ab')₂ anti-gp205 was a linear function of the input of labeled ligand and thus was nonsaturable and taken to be nonspecific (Fig. 3). In contrast, the binding of ^{125}I-F(ab')₂ anti-gp205 in the presence of preimmunization F(ab')₂ was concave to the abcissa, indicating saturable binding reactions for each of these cell types. The specific uptake of ^{125}I-F(ab')₂ was obtained by subtracting the amount bound nonspecifically from that bound in the absence of competing unlabeled ligand, and was plotted according to the method of Scatchard (36) to determine the total number of antigenic sites/cell. This analysis revealed 950 sites/E, 21,000 sites/cell of B lymphocyte preparation, 57,000 sites/PMN, and 48,000 sites/monocyte. Comparable analysis of T lymphocytes revealed no specific uptake of ^{125}I-F(ab')₂. Thus, the nucleated peripheral blood cells that are known to display C3b receptor activity had comparable numbers of membrane sites recognized by F(ab')₂ anti-gp205, whereas E had >10-fold fewer sites/cell and T lymphocytes, which are thought not to have C3b receptors, had no detectable antigenic sites.

Identification of the Membrane Protein of Human E, PMN, B Lymphocytes, and Monocytes that Reacts with IgG anti-gp205. In preliminary experiments, it was found that interaction of solubilized ^{125}I-labeled proteins of human E membranes with IgG anti-gp205 and *S. aureus* Cowan I precipitated 0.21% of the labeled proteins, whereas interaction of the membrane proteins with preimmunization IgG or with buffer and the bacteria precipitated 0.15% of the labeled proteins. To increase the percent of specifically immunoprecipitated ^{125}I-protein, the solubilized membrane proteins were subjected to affinity chromatography on Sepharose-C3 before interaction with anti-gp205. In two experiments, 0.40 and 0.58% of the applied protein-bound ^{125}I was recovered in the eluate of the column and 8.5 and 5.0%, respectively, of the eluted material was specifically immunoprecipitated. No labeled proteins in the effluent were specifically bound by IgG anti-gp205. Thus, partial purification of E membrane proteins by Sepharose-C3 chromatography resulted in an 80- to 140-fold increase in the percent of labeled proteins specifically interacting with IgG anti-gp205. The solubilized, radioiodinated membrane proteins from each of two preparations of PMN, B lymphocytes, and monocytes were also partially purified by Sepharose-C3 chromatography, and similar results were obtained. Only 0.1–0.7% of the applied
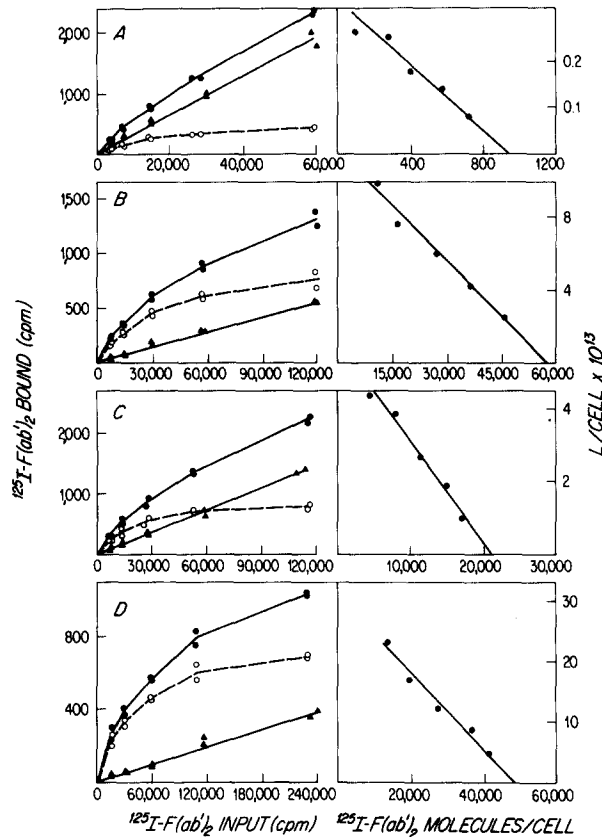
Fig. 3. Binding of ^{125}I-F(ab′)$_2$ rabbit anti-gp205 to human E (A), PMN (B), peripheral blood mononuclear cells enriched for B lymphocytes (C), and monocytes (D). In the left-hand portions of the panels, specific binding (O) of ^{125}I-F(ab′)$_2$ anti-gp205 was calculated from binding in the presence of preimmunization rabbit F(ab′)$_2$ (●) by subtracting the binding in the presence of excess, unlabeled F(ab′)$_2$ anti-gp205 (▲). The duplicate measurements at each input of ^{125}I-F(ab′)$_2$ are indicated. In the right-hand portions of the panels are Scatchard plots of the specific binding of ^{125}I-F(ab′)$_2$ anti-gp205 to each type of cell. Each point is the mean of the duplicate determinations.

protein-bound ^{125}I was recovered in the eluate of the Sepharose-C3 columns, and 4–10.4% of eluted proteins were specifically immunoprecipitated.

The immunoprecipitated proteins of each type of cell were subjected to SDS-PAGE and the 1-mm slices of the gels were assayed for ^{125}I. A single peak of ^{125}I with an apparent 205,000 mol wt was seen with material derived from each cell. Thus, IgG anti-gp205 recognized a single membrane protein of the human E, PMN, B lymphocyte, and monocyte that is identical in size with gp205.

Discussion

Initially, gp205 was sought and characterized as a constituent of human E membranes that could impair activation of the human alternative complement pathway by these cells (16). This glycoprotein of 205,000 mol wt was purified to homogeneity in its detergent-solubilized form and was shown to induce a dose-related loss of function of C3b,Bb,P by displacement of Bb from C3b, and to promote cleavage of

the α-polypeptide chain of C3b by C3bINA. These inhibitory activities of gp205, and its reversible adsorption to Sepharose-C3 during purification suggested that gp205 had an affinity for C3b and prompted the consideration that this membrane glycoprotein might have an additional function; namely, that of serving as the C3b receptor of human E.

The relation of gp205 to the C3b receptor of human E was examined by utilizing monospecific rabbit antibody to gp205. 2 μg/ml of the IgG fraction of serum taken from a rabbit that had been immunized with purified gp205 completely neutralized the capacity of gp205 to inhibit C3b,Bb,P sites on sheep E, whereas 25 times this concentration of rabbit IgG taken before immunization had no effect on this inhibitory activity of gp205, thereby indicating that the postimmunization IgG had specificity for gp205 (Fig. 1). Evidence for the monospecificity of this IgG anti-gp205 was obtained by demonstrating that it immunoprecipitated a single, ^{125}I-labeled protein of 205,000 mol wt, as assessed by SDS-PAGE, on interaction with membrane proteins from human E that had been surface radioiodinated (Fig. 4). Thus, the capacity of the F(ab')$_2$ fragment of this monospecific IgG anti-gp205 to inhibit in a dose-response manner the formation of rosettes between human E and sheep EC3b established gp205 as the C3b receptor of human E (Fig. 2). The additional finding that Scatchard analysis of the uptake of ^{125}I-F(ab')$_2$ anti-gp205 by human E revealed 950 antibody-binding sites/cell, is similar to the estimation of 1,400 C3b receptors/human E based on uptake of ^{125}I-C3 (37).

The identity of gp205 as the C3b receptor also of human peripheral blood PMN, B lymphocytes, and monocytes was suggested by the capacity of F(ab')$_2$ anti-gp205 to inhibit formation of rosettes by these cells with sheep EC3b (Fig. 2) without impairing the function of receptors for C3bi and C3d (38) on monocytes and B lymphocytes (Table I). However, seven- to ninefold higher concentrations of the antibody were required to inhibit by 50% C3b receptor-dependent formation of rosettes by these nucleated cells than was necessary for comparable inhibition of receptor function on human E. This difference was secondary to the presence of more C3b receptors on the
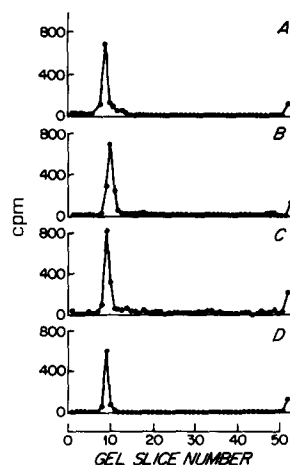

Fig. 4.   Analysis by SDS-PAGE of the ^{125}I-labeled membrane protein of human E (A), PMN (B), peripheral blood mononuclear cells enriched for B lymphocytes (C), and monocytes (D) that was immunoprecipitated by IgG anti-gp205 and protein A-bearing *S. aureus*.

nucleated cells as Scatchard analysis of the number of specific ^{125}I-F(ab′)$_2$ binding sites revealed 21,000 sites/cell of B lymphocyte preparation, 48,000 sites/monocyte, and 57,000 sites/PMN (Fig. 3). Because the preparation of B lymphocytes contained cells that lacked membrane Ig and that did not form rosettes with sheep EC3b, the number of anti-gp205-binding sites on B lymphocytes may be higher. Although the stoichiometry of the interaction of the bivalent, polyclonal anti-gp205 with its membrane antigen is not known, this analysis suggests that each of these nucleated cells may have 20- to 60-fold more C3b receptors than human E, and accounts for the higher concentrations of F(ab′)$_2$ anti-gp205 required for inhibition of C3b-receptor function on these cells, and for the capacity of these cells to bind 10–20 sheep EC3b, as compared to the 2–3 EC3b that are bound by human E. The absence of specific binding of ^{125}I-F(ab′)$_2$ by peripheral blood T lymphocytes is consistent with the inability of these cells to form rosettes with C3b-bearing particles.

Direct evidence for the identity of gp205 as the C3b receptor of PMN, B lymphocytes, and monocytes was obtained when radioiodinated, detergent-solubilized membrane proteins of these cells were reacted with anti-gp205 and the immunoprecipitate was analyzed by SDS-PAGE. IgG anti-gp205, in the presence of protein A-bearing *S. aureus*, specifically precipitated labeled material which was derived from each of these cell types, and which had been enriched by affinity chromatography on Sepharose-C3. In each instance, the immunoreactive material represented a single iodinated protein that exhibited an apparent 205,000 mol wt and was indistinguishable from the membrane protein derived from human E (Fig. 4). gp205, which was initially recognized as a membrane constituent of human E that is capable of inhibiting the alternative complement pathway, also serves as the C3b receptor of E, PMN, B lymphocytes, and monocytes.

Summary

A human erythrocyte membrane glycoprotein of 205,000 mol wt (gp205) has been identified as the C3b receptor of the erythrocyte, polymorphonuclear leukocyte (PMN), B lymphocyte, and monocyte. Initially, gp205 was sought and characterized as a constituent of the human erythrocyte membrane that can impair activation of the alternative complement pathway by inducing loss of function of the properdin-stabilized amplification C3 convertase (C3b,Bb,P) through displacement of Bb from C3b and by promoting cleavage-inactivation of C3b by C3b inactivator. These inhibitory activities of gp205 suggested that this membrane glycoprotein had an affinity for C3b and prompted an analysis of its possible identity as the C3b receptor of human peripheral blood cells. The F(ab′)$_2$ fragment of rabbit IgG anti-gp205 inhibited the formation of rosettes with sheep EC3b of human erythrocytes, B lymphocytes, monocytes and PMN in a dose-response manner; the 50% inhibitory doses were 0.13 μg/ml, 0.90 μg/ml, 1.25 μg/ml, and 1.20 μg/ml of F(ab′)$_2$, respectively. Anti-gp205 did not impair the formation of rosettes by monocytes and B lymphocytes with sheep EC3bi or with EC3d. Scatchard analysis of the number of specific ^{125}I-F(ab′)$_2$ anti-gp205 binding sites/cell revealed 950 sites/erythrocyte, 21,000 sites/cell of B lymphocyte preparation, 57,000 sites/PMN, and 48,000 sites/monocyte, indicating that the higher concentrations of antibody that had been required for inhibition of rosette formation by the nucleated cells reflected larger numbers of receptors on these cells. Direct evidence for the identity of gp205 as the C3b receptor of the four

cell types was obtained when detergent-solubilized membrane proteins of the surface-radioiodinated cells were reacted with anti-gp205 and the immunoprecipitate was analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. In each instance, the antigenic material reacting with anti-gp205 represented a single protein with an apparent 205,000 mol wt. Thus, gp205 is the C3b receptor of human erythrocytes, PMN, B lymphocytes, and monocytes.

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