Quinine-Dependent Antibodies to Neutrophils React With a 60-Kd Glycoprotein on Which Neutrophil-Specific Antigen NB1 Is Located and an 85-Kd Glycosyl-Phosphatidylinositol–Linked N-Glycosylated Plasma Membrane Glycoprotein

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We have previously described a 24-year-old woman with quinine-dependent antibodies that reacted with neutrophils, red blood cells (RBCs), platelets, and T lymphocytes. The drug-dependent neutrophil antibody was found to react with 85- and 60-Kd neutrophil membrane molecules. In these studies, we further characterized these molecules and found that both were glycosyl-phosphatidylinositol (GPI)-linked and contained sialic acid residues and N-linked carbohydrate side chains, but neither contained O-linked carbohydrates. The protein backbone of the 60-Kd molecule was 45 Kd, and the 85 Kd glycoprotein (GP) was made up of 33- and 31-Kd proteins. While some GPI-anchored neutrophil GPs are released by stimulated neutrophils, neither the 85- nor the 60-Kd GP was released by neutrophil stimulants. We have previously described a 24-year-old woman with quinine-dependent antibodies that reacted with neutrophils, platelets, and T lymphocytes. The quinine-dependent antibodies were absorbed with C5a, f-met-leu-phe (FMLP), or phorbol myristate acetate (PMA). Neutrophil-specific antigen NB1 is located on a 58- to 64-Kd GP. To determine if the quinine-dependent antibody and anti-NB1 recognize the same GP, immunoprecipitation studies were performed with the quinine-dependent antibody using neutrophils with varying NB1 phenotypes. The 60-Kd GP was detected on NB1-positive neutrophils from 11 of 12 donors tested, but not on NB1-negative neutrophils from two donors tested. After solubilized 122I-labeled neutrophils were absorbed with anti-NB1, the quinine-dependent antibody immunoprecipitated the 85-Kd GP, but not the 60-Kd GP. These results indicate that anti-NB1 and the quinine-dependent antibody identified the same GP. The 85-Kd GP was detected on neutrophils from all 14 donors tested. The electrophoretic mobility of the 85-Kd GP was similar to the electrophoretic mobility of the major 122I-labeled neutrophil protein.

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MATERIALS AND METHODS

Isolation of neutrophils. Whole blood was collected from donors of known neutrophil phenotype by peripheral vein phlebotomy into sterile glass tubes containing dry sodium EDTA (Terumo Medical, Elkton, MD) after obtaining informed consent. Neutrophils were isolated by dextran sedimentation and centrifugation over Ficoll-Hypaque

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mmol/L NaCl, 0.5% Nonidet P-40 [Sigma], 2 mmol/L phenylmethyl sulfonyl fluoride [PMSF, Sigma]), 0.02% NaN3, and 1% aprotinin (Sigma). The solution was incubated on ice for 60 minutes and insoluble material was removed by centrifugation at 10,000g for 30 minutes at 4°C. Antigens that react with normal human serum (NHS) were removed from the iodinated cell lysate by incubating 1 mL of cell lysate with 80 µL NHS at 4°C for 60 minutes. The resulting antigen-antibody complexes were immunoprecipitated by incubation with 600 µL of 10% protein A sepharose (wt/vol) (Pharmacia Fine Chemicals, Piscataway, NJ) in 100 mmol/L Tris pH 8.1, 150 mmol/L NaCl, 0.5% Nonidet P-40, 2 mmol/L PMSF, and 1% aprotinin at 4°C for 20 minutes. The solution was then centrifuged at 2,000g for 5 minutes at 4°C and the supernatant was treated with 300 µL of 10% protein A sepharose again as described above. To remove antigens recognized by normal rabbit serum (NRS), the cell lysate was also treated with 80 µL NRS as described above.

Radiolabeled antigens were immunoprecipitated from 100 µL of cell lysate by incubation with 20 µL of polyclonal antisera or 20 µL of antisera plus 20 µL of quinine hydrochloride (0.2 mg/mL in PBS) at 4°C overnight. Fifty microliters of 10% protein A sepharose in lysis buffer containing 0.1% bovine serum albumin (BSA; Sigma) was added, and after a 20-minute incubation at 20°C, the solution was centrifuged at 2,000g for 5 minutes at 4°C. The resulting pellet was washed twice in a buffer containing 0.5% deoxycholate, 1% aprotinin, then suspended in 100 pL of Laemmeli sample buffer (62 mmol/L Tris-HCl, pH 6.8, 2% sodium dodecyl sulfate [SDS]; BioRad Laboratories, Richmond, CA), 10% glycerol, and 0.001% bromophenol blue with or without 5% mercaptoethanol [2-ME; Sigma], incubated at 95°C for 5 minutes, and analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) in a 10% gel. The gel slabs were stained, dried, and examined by autoradiography. Molecular weight standards were: phosphorylase B, 97,400; BSA, 66,000; ovalbumin, 45,000; and carbonic anhydrase, 29,000 (Pharmacia).

GPI-PLC treatment of neutrophils. Neutrophils were labeled with 125I and suspended at a concentration of 2 x 10^7 cells/mL in PBS. One milliliter of the cell suspension plus 5 U of GPI-PLC (from Bacillus cereus; Boehringer Mannheim, Indianapolis, IN) was incubated for 60 minutes at 37°C and then centrifuged for 5 minutes at 500 x g. The pellet cells were washed once with PBS and resuspended in 1 mL of lysis buffer. The supernatant and pellet were analyzed by immunoprecipitation as described above.

Treatment of immunoprecipitated molecules with glycolytic enzymes. Neutrophils were labeled with 125I, solubilized, and reacted with antibodies and protein A as described above. The resulting antibody-antigen protein A sepharose complex was resuspended in 60 µL of a buffer containing 50 mmol/L EDTA, 1 mmol/L PMSF, 1% 2-ME, 1% Nonidet P-40, 0.1 mol/L Na2PO4 pH 7.5, and 0.1% SDS, and incubated at 95°C for 5 minutes. The final concentration of Triton-X-100 was brought up to 0.5% by adding 15 µL of 2.5% Triton-X-100. The solution was then incubated with 1.0 U of N-glycanase (Genzyme, Boston, MA) at 37°C for 20 hours. Reducing Laemmeli sample buffer (20 µL) was added and the sample was denatured by boiling at 95°C for 5 minutes, and centrifuged at 2,600g for 5 minutes at 4°C. The supernatant was analyzed by SDS-PAGE and autoradiography.

When the labeled molecules were treated with neuraminidase or O-glycanase, (both Genzyme), a similar procedure was used, except the antigen-antibody protein A sepharose pellet was resuspended in 60 µL of a buffer containing 15 mmol/L Tris-malate pH 6.15 and 0.1% SDS. This solution was boiled at 95°C for 5 minutes and then 15 µL of 2.5% Triton X-100 was added so that its final concentration was 0.5%. Either neuraminidase (acetylneuraminyl hydrolase from Streptococcus, 0.05 U) was added and the solution incubated for 1 hour at 37°C, or O-glycanase (4.0 mU) was added and the solution incubated for 20 hours at 37°C. When the immunoprecipitated molecules were tested with both enzymes, the labeled protein solution was treated with 0.05 U of neuraminidase for 1 hour at 37°C followed by 4 mU of O-glycanase and the incubation was continued for another 20 hours at 37°C. The immunoprecipitated GPs were then analyzed by SDS-PAGE and autoradiography. As a control, fetuin (Sigma) was treated with neuraminidase and O-glycanase and analyzed by SDS-PAGE; as expected, its electrophoretic mobility increased.

*Testing stimulated neutrophils for the expression of quinine-dependent antigens.* Neutrophils were labeled with 125I and suspended at a concentration of 2 x 10^7 cells/mL in HBSS. The cell suspensions were then incubated with f-met-leu-phe (FMLP; Sigma) 10^-7 mol/L, C5a (Sigma) 10^-7 mol/L, and phorbol myristate acetate (PMA; Sigma) 100 ng/mL at 37°C for 15 minutes. The suspensions were centrifuged at 500g for 5 minutes. The supernatant was centrifuged at 10,000g for 30 minutes and analyzed by immunoprecipitation. The pelleted cells were resuspended at a concentration of 2 x 10^7 cells/mL in cell lysate buffer and were incubated at 4°C for 30 minutes, centrifuged at 10,000g for 5 minutes, and the resulting supernatant analyzed by immunoprecipitation.

**RESULTS**

Immunoprecipitation with quinine-dependent antibodies using neutrophils with varying NB phenotypes. Our patient's quinine-dependent antibody reacted with a 60-Kd membrane molecule that could be on the same molecule that the NB1 antigen was located. Using GIF and GA assays, we have previously found that the patient serum plus quinine reacted with NB1-positive and -negative cells. However, since the patient serum contained multiple quinine-dependent antibodies, the possibility that an antibody to NB1 antigen was present could not be excluded. To determine if the 60-Kd molecule that the quinine-dependent antibody reacted with was the same molecule on which NB1 antigen was located, neutrophils from three donors with varying NB1 phenotype were surface-labeled with 125I, solubilized, and reacted with patient serum plus quinine. The serum plus quinine immunoprecipitated an 85-Kd molecule from all three donors (Fig 1, lanes A through C). Serum plus quinine reacted with a 60-Kd molecule on NB1-homozygous neutrophils (lane A) and NB1-heterozygous neutrophils (lane B), but did not detect the 60-Kd molecule on NB2-homozygous neutrophils (NB1-negative) (lane C). NHS did not immunoprecipitate any molecules of similar molecular weight from neutrophils from the same three donors (lanes D through F).

Immunoprecipitation studies with patient serum plus quinine were performed with neutrophils from 11 other donors. Overall, the 85-Kd molecule was detected on neutrophils from all 14 donors tested. The antibody plus quinine immunoprecipitated the 60-Kd molecule from 11 of 12 NB1-positive neutrophils, but the 60-Kd molecule was not detected on either of two NB1-negative neutrophils tested.

Sequential immunoprecipitation with anti-NB1 and quinine-dependent antibodies. To confirm that anti-NB1 and the quinine-dependent antibody recognized the same 60-Kd molecule, solubilized 125I-labeled neutrophils were absorbed three times with NHS or anti-NB1. Control neutrophils (Fig
The quantity of 85- and 60-Kd molecules immunoprecipitated from neutrophils absorbed with NHS was slightly less than that immunoprecipitated from control neutrophils. This was likely due to the dilution of labeled proteins during the absorption with NHS.

Because our patient experienced quinine-dependent neutropenia and in the presence of quinine her serum recognized the 60-Kd NB1 antigen-bearing molecule, her neutrophils were phenotyped for NB1 antigen. Her neutrophils reacted with reference anti-NB1 in both the GA and GIF phenotyping assays.

Effects of GPI-PLC on neutrophil expression of the molecules recognized by the quinine-dependent antibodies. Several neutrophil membrane proteins, including the NB1 molecule and FcRIII, are anchored to the neutrophil plasma membrane via a GPI-linkage. GPI-linked membrane proteins can be selectively cleaved from neutrophils by treatment with GPI-PLC. To determine if the 85-Kd molecule was GPI-linked, neutrophils were treated with GPI-PLC and immunoprecipitated with the quinine-dependent antibody. Neutrophils were surface-labeled with 125I using lactoperoxidase and incubated with PBS pH 7.4 or GPI-PLC

Fig 1. Immunoprecipitation with quinine-dependent antibodies of 125I surface labeled neutrophil membrane proteins prepared from neutrophils with varying NB phenotypes. NB1-homozygous neutrophils (lanes A and D), NB-heterozygous neutrophils (lanes B and E), and NB2-homozygous neutrophils (lanes C and F) were labeled with 125I, solubilized, and immunoprecipitated with patient serum plus quinine (lanes A through C) or NHS (lanes D through F). Immunoprecipitated GPs were analyzed by SDS-PAGE in a 10% gel under reducing conditions and autoradiography. Molecular weight standards were: phosphorylase B, 97,400; BSA, 66,000; ovalbumin, 45,000; carbonic anhydrase, 29,000.

Fig 2. Results of sequential immunoprecipitation studies performed with the quinine-dependent antibodies and NB1 antibodies. Neutrophils were surface-labeled with 125I, solubilized, and absorbed with NHS (lanes D through F) or anti-NB1 (lanes G through I). Immunoprecipitation was then performed with unabsorbed and absorbed neutrophil lysates with NHS (lanes A, D, and G), quinine plus patient serum (lanes B, E, and H), or anti-NB1 (lanes C, F, and I). Immunoprecipitated GPs were analyzed by SDS-PAGE under reducing conditions and autoradiography.
at 37°C for 60 minutes. The suspensions were then centrifuged at 500g for 5 minutes at 20°C. The resulting supernatant and pelleted cells were analyzed by immunoprecipitation with patient serum plus quinine and anti-NB1. As a control, immunoprecipitation was performed with NHS, patient serum plus quinine, and anti-NB1 with neutrophils, which were not incubated with PBS or GPI-PLC (Fig 3, lanes A through C). As expected, the quinine-dependent antibody reacted with the 85- and 60-Kd molecules (lane B), anti-NB1 reacted with a 60-Kd GP (lane C), and NHS did not react with any molecules (lane A). After neutrophils were incubated with PBS, patient serum plus quinine reacted with the 85- and the 60-Kd molecules on the pelleted cells (lane E), but not in the supernatant (lane H), and anti-NB1 detected a 60-Kd GP on the cells (lane F), but not in the supernatant (lane I). Following treatment with GPI-PLC, patient serum plus quinine did not detect the 85-Kd molecule, but reacted weakly with the 60-Kd molecule on the pelleted neutrophils (lane K). Both the 85- and 60-Kd molecules were detected in the supernatant (lane N). As expected, after treatment with GPI-PLC, anti-NB1 reacted strongly with a 60-Kd GP in the supernatant (lane O), and weakly with the 60-Kd GP on pelleted cells (lane L). These results confirm previous studies which found that most, but not all, of the 60-Kd molecule on which NB1 antigen was located was anchored to neutrophil plasma membranes via a GPI-linkage. These studies also show that the 85-Kd molecule identified by the quinine-dependent antibody is GPI-anchored.

Treatment of the 85-Kd and 60-Kd molecules with glycolytic enzymes. Many blood cell membrane proteins contain N-linked or O-linked carbohydrate side chains. We have previously shown that the molecule on which NB1 antigen is located is N-glycosylated and the molecular weight of the molecule after removal of N-linked carbohydrates was 45 Kd. To determine if the 85-Kd molecule was also glycosylated, the molecules immunoprecipitated by patient serum plus quinine were treated with N-glycanase, O-glycanase, O-glycanase plus neuraminidase, and neuraminidase alone and analyzed by SDS-PAGE and autoradiography.

First, neutrophils from the same donor shown in Fig 3 were surface-labeled with 125I and immunoprecipitated with NHS, patient serum plus quinine, or anti-NB1. The immunoprecipitated molecules were then treated with N-glycanase at 37°C for 24 hours and analyzed by SDS-PAGE and autoradiography. After the 85- and 60-Kd molecules immunoprecipitated by the quinine-dependent antibody were treated with N-glycanase, 45-, 33-, and 31-Kd proteins were detected (Fig 4, lane B). After the 60-Kd molecule immunoprecipitated by anti-NB1 was treated with N-glycanase, only the 45-Kd protein was detected (lane C). When molecules immunoprecipitated with NHS were reacted with N-glycanase, the 45-, 33-, and 31-Kd proteins were not detected (lane A). These results suggest that the 33- and 31-Kd proteins make up the 85-Kd molecule. To confirm this, the above studies were repeated with neutrophils that did not express NB1 antigen. The 85-Kd molecule, but not the 60-Kd mol-

![Fig 3. The 85- and 60-Kd molecules recognized by the quinine-dependent antibody are GPI-linked. NB-heterozygous 125I-labeled neutrophils were immunoprecipitated with NHS (lane A), patient serum plus quinine (lane B), or anti-NB1 (lane C) and analyzed by SDS-PAGE and autoradiography. NB heterozygous neutrophils were labeled with 125I and incubated at 37°C for 60 minutes with PBS (lanes D through I) or GPI-PLC (lanes J through O), and then centrifuged at 500g for 5 minutes. The pelleted neutrophils were solubilized and the supernatants and solubilized cells were analyzed by immunoprecipitation with NHS (lanes D, G, J, and M), patient serum plus quinine (lanes E, H, K, and N), or anti-NB1 (lanes F, I, L, and O). Immunoprecipitated GPs were analyzed by SDS-PAGE under reducing conditions and autoradiography.](https://www.bloodjournal.org/content/2761/1/2761/F3?cylkmode=true&cylkresmode=true&cylksize=1000)
To determine if the 85- and 60-Kd GPs immunoprecipitated by quinine-dependent antibodies contained O-linked carbohydrates, they were treated with O-glycanase. Because N-acetyl-neuraminic (sialic acid) residues may have to be removed for O-glycanase to cleave effectively O-linked carbohydrates, the 85- and 60-Kd GPs were treated with both O-glycanase and neuraminidase. NB1-positive neutrophils were surface-labeled with $^{125}$I and immunoprecipitated with patient serum plus quinine. The immunoprecipitated material was then incubated with buffer, neuraminidase, neuraminidase plus O-glycanase, or O-glycanase alone at 37°C for 24 hours and analyzed by SDS-PAGE and autoradiography. The results of the enzyme treatment of the 85- and 60-Kd GPs are shown in Fig 5. Glycoproteins that were immunoprecipitated by the patient serum plus quinine and incubated with buffer alone are shown in lane A. Following treatment with neuraminidase, the electrophoretic mobility of the 85- and 60-Kd GPs decreased in size slightly (lane B). When 85- and 60-Kd GPs were treated with both neuraminidase and O-glycanase, there was no difference in electrophoretic mobility of either GP compared with the GPs treated with neuraminidase alone (lane C). O-glycanase alone had no effect on either the 85- or 60-Kd GP (lane D). An approximately 60-Kd band is seen in lanes B and C. This label represents the displacement of labeled proteins in the gel by the neuraminidase. Neutrophils from three other donors were...
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also treated with neuraminidase and the results were similar. These results indicate that both the 60- and 85-Kd GPs immunoprecipitated by patient serum plus quinine contained sialic acid residues, but neither contained O-linked carbohydrates.

Comparison of the electrophoretic mobility of the GPs immunoprecipitated by quinine-dependent antibodies and neutrophil proteins labeled by \(^{125}I\). When immunoprecipitation studies were performed with patient serum plus quinine, the intensity of immunoprecipitated 85-Kd GP always appeared greater than the intensity of the 60-Kd GP. Others have found that the most prominently \(^{125}I\)-labeled surface-labeled neutrophil GP (major labeled protein) was a 60- to 90-Kd molecule that was GPI-linked.\(^1\) To determine if the 85-Kd molecule immunoprecipitated by patient serum plus quinine and whether the major labeled protein might be the same protein, the electrophoretic mobility of the molecule immunoprecipitated by the quinine-dependent antibody and the major labeled protein were compared. Electrophoretic mobility of the immunoprecipitated 85-Kd GP (Fig 6, lane A) was similar to the electrophoretic mobility of the major labeled protein (lane B).

Effects of stimulation on the expression of the molecules identified by the quinine-dependent antibodies. The GPI-linked FcRIII is released from plasma membranes when neutrophils are stimulated.\(^7\) To determine if the 85- and 60-Kd GPs were released by stimulated neutrophils, \(^{125}I\)-labeled neutrophils were stimulated with PMA, C5a, or FMLP, centrifuged, and the supernatants and pelleted cells were analyzed by immunoprecipitation with serum plus quinine and anti-NB1. Following stimulation of neutrophils with C5a or FMLP, the 85- and 60-Kd GPs were detected on neutrophils, but not in the supernatant (Fig 7). Some NB1 antibodies immunoprecipitate an approximately 80-Kd GP in addition to the 60-Kd GP. The 80-Kd GP was detected weakly on pelleted cells and strongly in the supernatants of neutrophils incubated in buffer, FMLP, or C5a. Similar results were obtained when neutrophils were stimulated with PMA (data not shown).

Immunoprecipitation with quinine-dependent antibodies that had been absorbed with RBCs or platelets. We have previously found that in the presence of quinine, the patient serum reacted RBCs and platelets.\(^5\) After patient serum plus quinine was absorbed with RBCs or platelets, the antibody continued to react with neutrophils, indicating that at least one of the epitopes recognized by the quinine-dependent neutrophil antibodies was not expressed on platelets or RBCs.\(^5\) While NB1 antigen is not expressed on RBCs or platelets, to determine if the epitope on the 85-Kd GP recognized by the quinine-dependent antibody was present on platelets or RBCs, patient serum that had been absorbed with RBCs or platelets was tested in the immunoprecipitation assay. Both the RBC and platelet absorbed serum continued to immunoprecipitate the neutrophil 85-Kd GP (data not shown).

Analysis on the GPs immunoprecipitated by the quinine-dependent antibodies by SDS-PAGE under nonreducing conditions. When the 85-Kd GP immunoprecipitated by the quinine-dependent antibody was treated with N-glycanase, two proteins were detected. When the 85-Kd GP immunoprecipitated by patient serum plus quinine was analyzed by SDS-PAGE under reducing conditions and the gel was exposed for varying periods of time, only one 85-Kd GP was detected.

Because the electrophoretic mobility of some GPs change when analyzed under nonreducing conditions, to test further for the presence of two separate 85-Kd GPs, the immunoprecipitated GPs were analyzed by SDS-PAGE run under reducing and nonreducing conditions (Fig 8). When the GPs were analyzed under nonreducing conditions, the 85-Kd GP continued to appear to be a single GP (lane A). Although the electrophoretic mobility of the 85-Kd GP was similar when analyzed under nonreducing conditions, the 85-Kd GP continued to appear to be a single GP (lane A). Although the electrophoretic mobility of the 85-Kd GP was similar when analyzed under nonreducing conditions, the 85-Kd GP continued to appear to be a single GP (lane A). Although the electrophoretic mobility of the 85-Kd GP was similar when analyzed under nonreducing conditions, the 85-Kd GP continued to appear to be a single GP (lane A).

DISCUSSION

The quinine-dependent antibodies in our patient reacted with an 85-Kd and a 60-Kd GP, both of which were GPI-
linked and which contained sialic acid residues and N-linked but not O-linked carbohydrate side chains. The 60-Kd GP recognized by the quinine-dependent antibody was the same GP on which NB1 antigen was located. While anti-NB1 and the quinine-dependent antibody recognized the same GP, the quantity of the 60-Kd GP immunoprecipitated by anti-NB1 was greater than the quantity immunoprecipitated by the quinine-dependent antibody. This could have been due to weaker reactions to the 60-Kd GP by the quinine-dependent antibody compared with anti-NB1. However, NB1 antigen is expressed heterogeneously on neutrophils, and we cannot exclude the possibility that, compared with NB1 antigen, the epitope on the 60-Kd GP recognized by the quinine-dependent antibody may be present on a smaller population of neutrophils.

The expression of biallelic platelet antigens PL^A1 and PL^A2 are due to a single amino acid difference in platelet membrane GP IIa. While many quinine-dependent antibodies to platelets react with GPIIb/IIa, no quinine-dependent antibodies have been described that show specificity for PL^A1 or PL^A2 antigens. Neutrophil-specific antigen NB2 has been described as the allele of NB1; however, anti-NB2 has not been found to immunoblot or immunoprecipitate neutrophil membrane GPs and the biochemical structure of NB2 is not known. The reason why the quinine-dependent antibody reacted with NB1 but not NB2 antigen is not certain.

Neutrophil antigens NA1, NA2, and Mart have been biochemically characterized and are not located on the 85-Kd GP recognized by the quinine-dependent antibody. Neutrophil-specific antigens NA1 and NA2 are located on 50- to 65-Kd and 65- to 80-Kd FcRIII molecules. Neutrophil antigen Mart is located on the 165-Kd a M-integrin chain (CD11b). We cannot exclude the possibility that the 85-Kd GP and the protein most prominently labeled with ^bq are the same molecule.

These results confirmed the results of a previous study which found that the electrophoretic mobility of the 60-Kd NB1 GP was increased when analyzed by SDS-PAGE under nonreducing conditions compared with reducing conditions, suggesting that disulfide bands contributed to the structure of the NB1 molecule. The lack of change in the electrophoretic mobility of the 85-Kd GP when analyzed by SDS-PAGE under reducing and nonreducing conditions suggested that disulfide bonds contribute less to the structure of this molecule.

Interestingly, when the 85-Kd GP was treated with N-glycosidase, two separate proteins were detected, suggesting that the 85-Kd molecule may be made up of two separate but similar GPs. Both are GPI-linked and are N-glycosylated to a similar degree. It is possible that the 85-Kd molecule is made up of two GPs that have protein cores that differ by only a few amino acids. The FcRIII molecule on neutrophils that express both NA1 and NA2 antigen appears to be a single GP of 85 Kd. However, when the GP was treated with N-glycosidase, 29- and 33-Kd proteins were detected. The 29-Kd protein was encoded by the NA1 FcRIII gene and the 33-Kd protein was encoded by the NA2 FcRIII gene. The amino acid sequences of NA1 FcRIII and NA2 FcRIII differed in only four places.

We have previously shown that the quinine-dependent antibodies stimulated neutrophils. If, like neutrophil FcRIII, the 85- and 60-Kd GPs were released from stimulated neutrophil, soluble immune complexes could have formed in our patient and contributed to her renal failure, but neither GP was released from stimulated neutrophils. Goldschmeding et al have also found that NB1 antigen is not released from neutrophil stimulated with FMLP. Some NB1 antibodies immunoprecipitated an 80-Kd GP in addition to the 60-Kd NB1 GP. The current and a previous study found that this 80-Kd GP molecule was released from neutrophils during
incubation in buffer. While the quinine-dependent antibody reacted with the 60-Kd NB1 GP, it did not react with the 80-Kd GP.

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Quinine-dependent antibodies to neutrophils react with a 60-Kd glycoprotein on which neutrophil-specific antigen NB1 is located and an 85-Kd glycosyl-phosphatidylinositol-linked N-glycosylated plasma membrane glycoprotein

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