The surface glycoprotein CD36 (GPIV) is known to mediate the adhesion of Plasmodium falciparum malaria-infected red blood cells and to be a receptor for extracellular matrix proteins such as collagen and thrombospondin. The murine monoclonal IgM antibody NL07, which is specific for CD36, has now been shown to also be a potent inhibitor of the adherence of P. falciparum malaria-infected red blood cells to C32 melanoma cells. Treatment of platelets with NL07 monoclonal antibody resulted in rapid degranulation, release of ATP and serotonin, increase in [Ca²⁺], and tyrosine phosphorylation of a substrate protein of 130 kDa. In about one-half of the experiments, activation with NL07 resulted in the formation of small aggregates of 10 to 30 platelets, whereas in the other half of the experiments, large aggregates were seen similar to those induced by adenosine diphosphate (ADP) and these large aggregates could be converted to the small aggregates by ATPαS or by AP-2 or other antibodies against GPIb and/or Ila. Microaggregates of 2 to 5 platelets were seen with Ganzmann's platelets that constitutively lack GPIb/IIa. Aggregate formation was not seen with heat-treated serum, in the presence of anti C1q antibodies, or when using C5-, C8-, or C9-deficient human sera. Although activation of platelets with purified complement components results in a slow morphologic change without aggregation, involvement of CD36 results in rapid complement-mediated activation leading to formation of small aggregates that is largely independent of GPIb/IIa and that, under certain circumstances, proceeds to the formation of large ADP-dependent aggregates.

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phorylation. This activation results in the rapid formation of platelet aggregates that are smaller than those seen with the usual aggregating agents; their formation appears to be largely independent of GPIIb/IIIa, but under certain circumstances they can proceed to become large adenosine diphosphate (ADP)-dependent aggregates.

MATERIALS AND METHODS

The following reagents were obtained from Sigma Chemical Co (St Louis, MO): ADP; adenosine triphosphate (ATP); arachidonic acid; the thromboxane mimetic U66362H; ionophore A23187; acetysalicylic acid; phorbol myristate acetate (PMA); fibrinogen; RGDS peptide: 1-(5-isouquinolinesulfonyl)-2-methylpiperazine dihydrochloride (H7); quercetin; erastatin; sodium nitroprusside; pepsin; human thrombin (2,000 NIH units/mg of protein); lactate dehydrogenase (LDH) assay kit; UV-test and ATP assay mix; and C5-, C8-, and C9-deficient human sera. Collagen was from Chronolog Corp (Havertown, PA). RDGS peptide was from Sclavo (Siena, Italy). CD36 was isolated from outdated platelet concentrates (as previously described). Fab and F(ab), fragments, both lacking any detectable Fc portion, were obtained from NL07 by means of pepsin digestion, as previously described. Thrombospondin was from Diagnostica Stago (Asnières, France). Fura-2-AM was from Calbiochem (San Diego, CA). 1-Octadecyl-2-acetyl-glycero-3-phosphocholine (platelet activating factor [PAF]) was from Bachem (Bubendorf, Switzerland). Of the PAF inhibitors, Ro was from Hoffmann-La Roche (Basel, Switzerland), whereas CV6209 and CV3988 were from Takeda Chemical Co (Osaka, Japan). Trifluoroperazine was from Boehringer-Mannheim (Mannheim, Germany). Protein tyrosine kinase (PTK) inhibitor ST2809 was kindly provided by P.M. Comoglio (University of Turin, Italy). 125I-protein A was from Amersham (Buckinghamshire, UK). CD36 was isolated from outdated platelet concentrates as previously described.

Antibodies used in the study. NL07 was prepared as previously described. Fab' and F(ab)', fragments, both lacking any detectable Fe portion, were obtained from NL07 by means of pepsin digestion, following the technique previously reported with modifications. Briefly, purified NL07 was dialyzed against sodium acetate buffer (Na acetate at 20 mmol/L, NaCl at 150 mmol/L, pH 4.3) and then treated with pepsin at a protein: pepsin ratio of 30:1 (wt/wt) for 1 hour at 37°C. The reaction was stopped by bringing the pH to 7.0 by means of 1 mol/L Tris-HCl, pH 8.0. The solution was concentrated with a 50-kD cut-off membrane to rule out the presence of low molecular weight peptides. The fragments were then separated on a high performance liquid chromatography (HPLC) size-exclusion column (LKB Ultratrac TSK-G300 SWG, 21.5 x 600 mm) and finally tested by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for purity and by immunofluorescence for reactivity.

The following MoAbs were used in the present study: OKM5 and OKM8 (anti-CD36; Ortho, Raritan, NJ), AC4.25 (anti-β, microglobulin (β2m) and 01.65 (anti-HLA class I),33 CB04 (anti-C3 complement receptor that does not react with platelets),34 M753 directed against GPIIIa (Dakopatts A/S, Glostrup, Denmark), AP-2 against GPIIIb-IIIa (kindly provided by Thomas J. Kunicki, Scripps Clinic, La Jolla, CA), and antiphosphotyrosine (Upstate Biotechnology Inc, Lake Placid, NY). Rabbit polyclonal antisera #778 and #777 specific for human C1q were produced in the Torino laboratory. Rabbit polyclonal antibody anti-GPIIIa was kindly provided by G. Tarone (University of Torino). Goat antimouse Ig labeled with fluorescein isothiocyanate (GoMlg-FITC) was from Tecnogenetics (Milan, Italy).

Inhibition of cytoadhesion of parasitized RBCs (PRBCs). The adhesion of malaria-infected erythrocytes (ITG-CD36 strain) to C32 melanoma cells and to purified CD36 was performed as previously described. Briefly, 2 x 10^6 malaria cells were plated onto 24-well tissue culture plates. CD36 (0.25 μg/mL) was adsorbed to polystyrene bacteriologic dishes overnight at 4°C. MoAbs were added for 45 minutes at room temperature followed by two washes with RPMI-1640. Malaria-infected erythrocytes (20% to 40% parasitemia; 1% hematocrit) were added for 1 hour at room temperature. Unbound erythrocytes were removed by three washes with RPMI-1640 and the preparation was fixed with 2% glutaraldehyde. Cells were stained with Giemsa and the number of malaria-infected erythrocytes/100 melanoma cells or number of PRBCs bound per square millimeter were enumerated.

Platelet preparation. Platelets were prepared from blood of healthy donors. The blood sample was drawn with a butterfly-19 needle without using a tourniquet and the blood was collected dropwise into plastic tubes containing 3.8% Na-citrate (1:10 vol/vol) kept under gentle stirring. The blood was centrifuged at 100 g for 20 minutes at 20°C to sediment erythrocytes and mononuclear cells. The platelet-rich plasma (PRP) was used directly as prepared. Platelet-poor plasma (PPP) was obtained by centrifuging PRP in an Eppendorf microfuge (3 minutes at room temperature).

Blood from a well-characterized Glanzmann's thrombasthenia patient whose platelets contain ~10% of normal levels of GPIIb/IIIa was kindly provided by Robert Abel (Christiana Hospital, Dover, DE). The NAK,-negative donor (ARC-36) has been previously described.

Platelet aggregation and secretion. Platelet aggregation was measured in an Elvi 840 aggregometer (Elvi Logos, Milano, Italy) as percent increase in light transmission after the addition to PRP of agonist (or agonist plus inhibitor). PRP (250 μL) was added to magnetically stirred cuvettes and incubated at 37°C. The PRP was treated with purified NL07 or control MoAbs at final concentrations of 4 μg/mL, at 10 μmol/L for ADP, at 1 mmol/L for arachidonate, at 2.5 μg/mL for collagen, at 5 μg/mL for A23187, and at 10 μmol/L for PAF. Incubation times and concentrations of different inhibitors are indicated in the Results.

ATP secretion from platelet storage granules was measured by a luciferin-luciferase assay. Briefly, 250 μL of PRP (3 x 10^9/mL) was
Fig 1. Concentration-dependent platelet activation by various concentrations of NL07: (A) changes in light transmittance in PRP; (B) ATP release in PRP; (C) NL07-induced changes in light transmittance (----) and ATP release (-----) in heat-treated (56°C for 30 minutes) plasma. Untreated or treated serum or plasma at 200 μL was mixed with 200 μL of 2 x 10^5 platelets/mL. Collagen was used as a positive control in heat-treated samples to measure aggregation. An isotype-matched control MoAb AC 4.25 (4 μg/mL) neither activated the platelets nor blocked activation by NL07. The light transmission measured in a lumiaaggregateometer ranged from 0% with untreated PRP to 100% using PPP.

added to magnetically stirred cuvettes and incubated at 37°C. One minute after addition of the agonist to the sample, 2 μL of stimulated PRP was immediately transferred to a vial containing 300 μL of ATP assay mix. The luminescence was measured in a Magic Lite Analyzer (Ciba-Corning, Medfield, MA). ATP secretion was assessed in untreated platelet suspensions (negative controls), whereas maximal secretion (100%) was measured on arachidonate-treated platelets. The quantity of ATP released after specific stimulus was reported as a percentage of maximal secretion.

In other experiments, aggregation and ATP secretion were measured simultaneously in a Lumiaggregometer (Chronolog Corp, Havertown, PA). Inhibitory antibodies were incubated with 400 μL of PRP. Twenty-five microliters of luciferin-luciferase ATP assay mixture (Dupont), prepared in Tyrode’s-HEPES buffer, was added immediately before placing the cuvette in the instrument and adding the agonist. Levels of released ATP were determined from a standard curve using known amounts of ATP (2 to 10 μmol/L). Secretion was also measured by release of 3H-serotonin.36

Platelet fluorescence staining. Immunofluorescence was performed on citrated PRP with added heparin to avoid aggregation. Platelets in suspension (1 x 10^6) were reacted with appropriate (1:200 to 1:1,000) dilutions of ascites or purified MoAb (1 mg/mL) for 30 minutes at 4°C. Bound antibody was shown by goat secondary antibody (GaMlg-FITC). The cells were then analyzed on a FACScan cytofluorograph (Becton Dickinson, Mountain View, CA).

Antiphosphotyrosine protein blot. A 250-μL aliquot of PRP was treated with NL07 or the irrelevant MoAb CB04 (4 μg/mL), in the presence or absence of the PTK inhibitor ST280 (0.1 mmol/L). After 2 minutes of incubation, platelets were sedimented in an Ep-
pendorf minifuge (2 minutes) and incubated for 8 minutes at 100°C with SDS-PAGE sample buffer without glycerol and β-mercaptoethanol (2.5% SDS, 65 mmol/L Tris-HCl at pH 6.8). After reconstitution in the same buffer with 10% glycerol and 5% β-mercaptoethanol, the samples were centrifuged in an Eppendorf minifuge (15 minutes) and then electrophoresed on a 10% acrylamide SDS-PAGE as described elsewhere.13 Proteins resolved in SDS-PAGE were electro-transferred (2 hours at 60 V) to nitrocellulose sheets (Schleicher & Schuell, Dassel, Germany). The nitrocellulose sheets were then saturated for 12 hours at 4°C in TBS buffer (10 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl) in the presence of 5% bovine serum albumin (BSA). After rinsing with TBS, blots were incubated for 2 hours with antiphosphotyrosine MoAb (1:1,000 in TBS with 3% BSA), washed three times for 10 minutes each with TBS, and then incubated (90 minutes at room temperature) with 125I-protein A or with goat antimouse Ig (125I-GaMlg) that had been labeled by the chloramine-T method (300,000 cpm/mL in TBS with 3% BSA). After washing three times with TBS, nitrocellulose sheets were air-dried and exposed to autoradiography at −70°C.

Changes in cytoplasmic [Ca2+]i. Changes in intracellular Ca2+ were measured using the fluorescent probe Fura-2 by a previously described method with minor modifications. Briefly, platelets were labeled with Fura-2-AM (2.5 μmol/L at 37°C for 30 minutes) in PRP containing prostaglandin E1 (PGE1; 1 μg/mL) that had been adjusted to pH 6.5 with 1 mol/L citric acid and Fura-2-labeled platelets were then isolated by centrifugation. After plasma removal, platelets were resuspended in 1/40 volume of wash buffer before the addition of Tyrode's-HEPES buffer to achieve a platelet concentration of 2 × 10⁸/mL. The platelet suspension (450 μL) was mixed with 50 μL of autologous PPP before the addition of reagents. Maximum and minimal levels of fluorescence were determined in the presence of 50 μmol/L digitonin and 1 mmol/L Ca²⁺ (100% of Fura-2 fluorescence) and in the presence of 12 mmol/L EGTA in 12 mmol/L HEPES buffer (5% of Fura-2 fluorescence) and using a kd of 224 mmol/L at 37°C for Fura-2.

RESULTS

Inhibition of RBC cytoadherence. The number of malaria-infected RBCs bound per 100 C32 melanoma cells was reduced from 1,276 ± 240 in controls to 485 ± 40 by 100
Fig 4. FACS analysis of the expression of CD36 and HLA class I and class II molecules. Conventional indirect immunofluorescence tests were performed on PRP in the presence of heparin. NL07 MoAb (anti-CD36) (-----; curve A), 01.65 MoAb (anti-HLA class I) (----; curve B), AA3.84 MoAb (anti-HLA class II) (-----; curve C), and the control GaMIg-FITC (-----; curve D). FL: forward light, an indication of fluorescence intensity; FSC: forward scatter, which reflects variations in cell size; SSC: side scatter, which reflects variations in cell granule content.

ng/mL NL07 (Table 1). When used as positive controls, 1 µg/mL OKM5 reduced the number of adherent RBCs to 577 ± 80, whereas 1 µg/mL OKM8 reduced it to 477 ± 77. Direct attachment of infected RBCs to purified CD36 coated on polystyrene dishes showed that NL07 at 1 µg/mL reduced attachment from 2,555 ± 509 infected RBCs/mm² of coated surface to 1,166 ± 208, whereas the same concentration of OKM5 and OKM8 reduced it to 2,366 ± 350 and 1,950 ± 23, respectively.

Platelet aggregate formation and secretion. When examined in the aggregometer, all samples of platelets suspended in plasma or serum before treatment with NL07 gave the rapid changes in light transmittance associated with shape change and aggregation (Fig 1). However, when examined by light microscopy, about half of the samples were found to contain small aggregates of 10 to 30 platelets, whereas the remaining samples gave large aggregates similar to those seen with ADP (Fig 2); treatment of these large aggregates with ATP·P·i or with MoAb AP-2 directed against the GPIIb/IIIa complex converted the large aggregates to small aggregates but did not result in their complete dissociation. The formation of the small aggregates was not inhibited by ATP·P·S, by AP-2, by a rabbit polyclonal antibody against GPIIIa, or by dissociating the GPIIb/IIIa complex with EGTA at 37°C. The same donors were found to give either a small aggregate response or a large aggregate response on different occasions and even the same blood sample could give both responses in two separate determinations.

Aggregate formation was not seen after treatment of PRP with MoAb 4.25, an IgM directed against ε2-microglobulin that is highly expressed on platelets, or after treatment with an irrelevant IgM CB04. Furthermore, NL07-induced activation did not occur with Naka-negative platelets that constitutively lack CD36, nor was it seen with platelets suspended in heated (56°C for 30 minutes) plasma or serum, with washed platelets, with paraformaldehyde-fixed platelets, or in the presence of 5 mmol/L EDTA. Activation was not induced by either F(ab)’ or Fab fragments of NL07 and the addition of NL07 did not cause platelet lysis because extracellular LDH was not detected. The addition of throm-
Fig 5. Inhibition of NL07-induced platelet activation by specific protein kinase inhibitors. Platelets were incubated for 5 minutes with different concentrations of the PKC inhibitor H7 or for 30 minutes with different concentrations of the PTK inhibitor ST280 before adding NL07.

Bospondin (5 μg/mL), a putative ligand for CD36, did not affect the platelet response to NL07.

Incubation of platelets with a specific rabbit polyclonal anti-C1q antiserum (37°C for 10 minutes) reduced or abolished NL07-induced activation, whereas no effects were seen with nonimmune rabbit serum (Fig 3). Activation by NL07 was not supported when normal human platelets were suspended in C5-, C8-, or C9-deficient human serum.

NL07-induced activation was accompanied by ATP release in PRP, but not in heat-treated plasma or serum (Fig 1). It may be noted that the concentration of extracellular ATP detected after platelet activation with 4 μg/mL NL07 is 3 times (Fig 1C) to 8 times (Fig 1B) greater than the amount released by 2.5 μg/mL collagen. Release of ¹⁴C-serotonin was 29% ± 3% with NL07 (4 μg/mL) as compared with 55% ± 7% with collagen (2.5 μg/mL) in duplicate determinations performed on 2 days.

Flow cytometry. Platelets maintained in heparinized plasma or in phosphate-buffered saline solution did not show detectable aggregate formation. These platelets were subjected to cytofluorographic analysis using a panel of different MoAbs including 01.65 (anti-HLA class I), whose binding to platelets is quantitatively similar to that of anti-CD36 MoAb, and the isotype-matched AA3.84 (anti-HLA class II) (Fig 4).

Platelets in phosphate-buffered saline bound NL07 (curve A) and 01.65 anti-HLA class I (curve B) as measured by fluorescence intensity, but there were no changes in the forward or side scatter parameters indicative of morphologic changes arising from the binding. In contrast, changes in these parameters were seen with platelets suspended in heparinized plasma in the case of NL07, but not with the other antibodies, indicating that morphologic changes and degranulation had occurred under these conditions.

Effects of different inhibitors of signal transduction pathways on CD36-induced platelet activation. When platelets were incubated for 5 minutes with the specific PKC inhibitor H7 before the addition of NL07, light transmittance decreased in a dose-dependent manner, reaching a maximum inhibition of about 50% at 1 mmol/L (Fig 5), although the same concentration of H7 had little, if any, effect on aggregation induced by ADP (not shown). Incubation with the PTK inhibitor ST280 for 30 minutes before challenge with NL07 similarly reduced light transmittance by about 50% (Fig 5). Despite the fact that transmittance was reduced only 50%, aggregates were not seen in either of these samples. Treatment with H7 and ST280 together gave a reduction in transmittance to ~50%, the same as that seen after treatment with either reagent alone.

NL07-induced platelet activation was not affected by...
aspirin (1 mmol/L), indicating that the phospholipase A2/thromboxane pathway was not involved, nor by sodium nitroprusside, indicating the absence of an effect of nitric oxide or elevated guanylate cyclase. Similarly, no effects were seen with trifluoperazine, which inhibits platelet aggregation by binding calmodulin, nor with the PAF antagonists CV3988 and CV6209 (data not shown).

Antiphosphotyrosine Western blot. Western blot analysis using an antiphosphotyrosine MoAb showed that platelet activation by NL07 MoAb was followed by tyrosine phosphorylation of a substrate protein of 130 kD. This phosphoprotein did not appear in control platelets treated with irrelevant MoAb nor when platelets were stimulated with NL07 in the presence of the PTK inhibitor ST280 (Fig 6).

Glanzmann’s thrombasthenia platelets. Because the formation of small aggregates was not affected by antibodies directed against GPIIb/IIIa, additional studies were performed with Glanzmann’s thrombasthenia platelets that constitutively lack GPIIb/IIIa. Treatment of Glanzmann’s platelets in the aggregometer with NL07 and the changes in light transmission were similar to those seen with common agonists such as ADP, although the changes in light transmittance and ATP secretion were smaller (Fig 7). When examined by light microscopy, microaggregates comprising only 2 to 5 platelets were seen, whereas Glanzmann’s platelets treated with the IgM AC4.25 remained as single platelets (Fig 8).

Changes in \([\text{Ca}^{2+}]\). Basal \([\text{Ca}^{2+}]\) concentration in resting Fura-2-loaded platelets in a 10% plasma suspension containing 1 mmol/L external \([\text{Ca}^{2+}]\) was \(\sim 80\ \text{mmol/L}\) (Fig 9). Addition of ADP (A, 10 \(\mu\text{mol/L}\); curve 1) resulted in a rapid increase in \([\text{Ca}^{2+}]\), to a peak of \(\sim 200\ \text{mmol/L}\), with an immediate rapid decline that leveled off at slightly above basal levels. On the other hand, the addition of NL07 (A, 4 \(\mu\text{g/mL}\); curve 2) resulted in a lag phase of \(\sim 40\) seconds followed by a rapid increase in \([\text{Ca}^{2+}]\), that plateaued at \(\sim 200\ \text{mmol/L}\) and remained at that level until EGTA (1 mmol/L) was added at point B, when it decreased to baseline values within 10 seconds. If EGTA was added 1 minute before the addition of NL07 (A; curve 3), \([\text{Ca}^{2+}]\) remained at baseline values. NL07-induced elevations in \([\text{Ca}^{2+}]\) were completely blocked by prior addition of OKh45 (5 \(\mu\text{g/mL}\)). NL07-induced changes in \([\text{Ca}^{2+}]\), were identical in control platelets, in platelets pretreated with MoAb AP-2, and in Glanzmann’s thrombasthenia platelets (data not shown).

**DISCUSSION**

Platelet activation by purified complement components has been extensively studied, but much less appears to be known about complement-dependent processes induced by antibody binding to the platelet surface. With purified complement components, extensive studies by Sims and colleagues have shown that the membrane attack complex formed from purified C5b-9 causes the formation in the platelet membrane of a complement pore that may act as a channel for the passive exchange of \(\text{Ca}^{2+}\) and that leads to a series of activating events including transient and reversible membrane depolarization, increases in \([\text{Ca}^{2+}]\) and thromboxane \(A_2\), secretion of von Willebrand factor multimers, and the activation of protein kinase C and myosin light chain kinase.

In the present work, we have obtained similar changes as a result of the binding to platelets of NL07, an IgM MoAb against CD36. These changes included increased influx of \([\text{Ca}^{2+}]\), secretion of ATP and serotonin, and tyrosine phosphorylation of a substrate protein of 130 kD. It is obvious that complement plays a major role in platelet activation by NL07 because activation was not seen in the presence of anti-C1q antibodies nor when using C5-, C8-, or C9-deficient human plasma. The inability of acetylsalicylic acid to inhibit NL07-induced aggregation and ATP release is also consistent with complement (C5b-9) involvement. With NL07, as with purified complement components, extracellular LDH was not detectable, indicating that the complement-induced pore was of limited size and that platelet lysis had not occurred.

The finding that C5-, C8-, and C9-deficient sera did not support NL07-induced activation led us to re-examine our previous findings regarding activation by F(ab')\(_2\) and Fab fragments of NL07. When prepared by the procedure described in Materials and Methods, these fragments were
shown to be free of all undigested antibody and were unable to induce any detectable platelet aggregation alone or when cross-linked by an RoMlg serum (data not shown). These results further confirm that the biologic effects of NL07 are mediated through its complement binding domain.

However, there are major differences in the morphologic response of platelets activated with purified platelet components and those activated by NL07. In the former case, there was a slow reduction in light transmittance, arising from changes in platelet morphology, which reached a maximum at 10 to 15 minutes, and only single platelets were seen in the microscope with no evidence of aggregate formation. In contrast, with NL07, the reduction in light transmittance reached a maximum in the same time as ADP (2 to 3 minutes) and platelet aggregates were always seen as the result of NL07-induced platelet activation. These were either small aggregates of 10 to 30 platelets or large aggregates indistinguishable from those obtained with ADP, and these large aggregates were reversed to small aggregates in the presence of ATP or AP-2.

Because aggregate formation is not seen with purified C5b-9 even in the presence of added fibrinogen but is seen with NL07 in plasma or serum, it suggests that direct platelet-platelet contact is not sufficient and that some other plasma component(s) is involved. Although purified C5b-9 does not induce aggregation, dense granule ADP is released.
and aggregates are rapidly formed on the addition of exogenous ADP. It has been suggested that this failure of endogenous released ADP to cause aggregation is caused by rapid metabolism of the nucleotide during the relatively slow release induced by purified C5b-9. With NL07, the process of activation is so rapid that the concentration of released ADP could cause aggregation, as it does in the case of other agonists.

However, we have observed wide variations in the amount of detectable extracellular ATP after activation with NL07 and this variability may offer an explanation for the formation of ADP-dependent large aggregates, the formation of small aggregates resulting from the presence of ATP or AP-2, and interdonor and intradonor variability in aggregation response. The concentrations of ATP and ADP in the dense granules are approximately equal (~2.5 μmol/10^11 platelets) but slightly lower than the concentration of ATP in the cytosol (~3.6 μmol/10^11 platelets). ADP-dependent platelet responses. However, GPIIb/IIIa is apparently not involved in the formation of small aggregates because AP-2 has no effect and is clearly not involved in the formation of microaggregates because this occurs even with Glanzmann’s thrombasthenia platelets that contain only 10% of normal amounts of GPIIb/IIIa. The plasma and/or platelet membrane components mediating these two latter interactions remain to be determined.

It appears likely that two interrelated pathways are involved in NL07-induced platelet activation. One pathway is induced by the actions of the late complement components as previously described and results in changes in light transmission without aggregation. The second pathway appears to be induced by the binding of NL07 to CD36 and leads to formation of small aggregates that, under certain circumstances, can react with released ADP to give the large aggregates characteristic of GPIIb/IIIa-mediated platelet aggregation. We have previously shown that one function of CD36 is to accelerate the adhesion of platelets to collagen, although the mechanism for this acceleration is not known. The binding of NL07 to CD36 appears to exert a similar acceleratory function on complement-mediated platelet activation. This acceleration may be due to the fact that CD36 has been shown to be associated with the src-related protein tyrosine kinases fyn, lyn, and yes in resting platelets.

Activation of platelets by purified C5b-9 has been shown to result in the phosphorylation of 40-kD and 20-kD substrates because of the activation of protein kinase C and myosin light chain kinase. In the present work, we have shown the tyrosine phosphorylation of a 130-kD substrate and have shown that the formation of small aggregates is not seen in the presence of the protein kinase C inhibitor H7 or the tyrosine kinase inhibitor ST280 and that with ST280 the formation of the 130-kD phosphotyrosine phosphoprotein is suppressed further, suggesting a role for the tyrosine kinases in NL07-induced activation.

RBCs infected with P. falciparum malaria bind to CD36 present on cell surfaces, or to purified CD36 coated on polystyrene dishes, and this binding is reversed by the anti-CD36 IgG MoAbs OKM5 and OKM8. We have examined whether NL07 similarly inhibits the attachment of PRBCs. NL07 was a potent inhibitor of the binding of P. falciparum-infected RBCs to C32 melanoma cells and was, in fact, about 10-fold more potent than OKM5 and OKM8; for example, OKM5 and OKM8 at 1 μg/mL reduced the number of bound cells by about 60%, whereas the same degree of inhibition was achieved by 100 ng/mL NL07. NL07 was similarly more potent than OKM5 and OKM8 in inhibiting the attachment of PRBCs to a polystyrene surface coated with purified CD36.

These studies show that NL07 resembles other anti-CD36 MoAbs in its ability to inhibit the binding of malaria-infected RBCs. NL07 also induces rapid formation of small platelet aggregates by a process that appears to involve both complement and CD36-mediated pathways. This process is largely independent of GPIIb/IIIa, but under certain circumstances these small aggregates may react with released ADP to form the large aggregates characteristic of other platelet agonists.

ACKNOWLEDGMENT

Thanks are given to Dr M. Geuna for excellent assistance in the FACS experiments and to Dr M. Mariani for helpful suggestions regarding pepsin digestion of IgM molecules. The light photomicrographs of Glanzmann's platelets were kindly taken by Dr Christian Haudenschild.

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Platelet activation and inhibition of malarial cytoadherence by the anti-CD36 IgM monoclonal antibody NL07

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