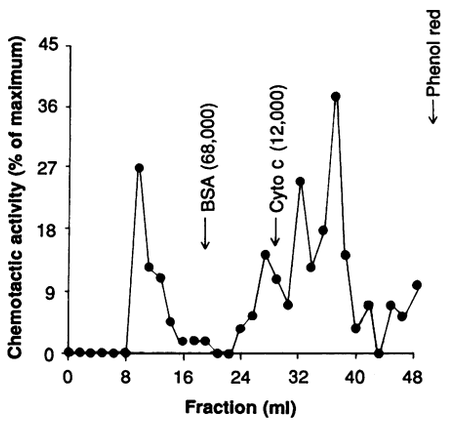

Fig. 4. Molecular size profile (in daltons) of TNF-α–induced endothelial cell supernatant chemotactic activity. Endothelial culture media were collected after 24 hours of treatment with TNF-α (20 ng/ml) or from unstimulated cultures. Particulate material was removed by centrifugation, concentrated fivefold, and fractionated by HPLC in a TSK-3000 SW (Varian Instruments) column. Fractions (1.6 ml) were collected and chemotactic activity was determined, as described in the legend to Fig. 2. The results are expressed as a percentage of the chemotactic activity (minus unstimulated control) induced by FMLP ($10^{-7}M$) and are representative of four individual studies. Abbreviations: BSA, bovine serum albumin; and cyto c, cytochrome c.

provide a signal to elicit selective diapedesis of neutrophils into the extravascular space.

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13 September 1988; accepted 9 December 1988

Identification of a Platelet Membrane Glycoprotein as a Falciparum Malaria Sequestration Receptor

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Infections with the human malaria parasite *Plasmodium falciparum* are characterized by sequestration of erythrocytes infected with mature forms of the parasite. Sequestration of infected erythrocytes appears to be critical for survival of the parasite and to mediate immunopathological abnormalities in severe malaria. A leukocyte differentiation antigen (CD36) was previously suggested to have a role in sequestration of malaria-infected erythrocytes. CD36 was purified from platelets, where it is known as GPIV, and was shown to be a receptor for binding of infected erythrocytes. Infected erythrocytes adhered to CD36 immobilized on plastic; purified CD36 exhibited saturable, specific binding to infected erythrocytes; and purified CD36 or antibodies to CD36 inhibited and reversed binding of infected erythrocytes to cultured endothelial cells and melanoma cells in vitro. The portion of the CD36 molecule that reverses cytoadherence may be useful therapeutically for rapid reversal of sequestration in cerebral malaria.

ERYTHROCYTES INFECTED WITH MATURE stages of the human malaria parasite *P. falciparum* are found sequestered along venular endothelium and not in the peripheral circulation of patients with malaria (*1*). Sequestration appears to contribute to the immunopathology of malaria (*2, 3*) and to the survival of the parasite by protecting nondeformable infected erythrocytes from destruction in the spleen (*4*). Assays of infected erythrocyte cytoadherence to cultured endothelial cells or C32 melanoma cells have been developed as in vitro correlates of sequestration (*5, 6*). Electron-dense knobs that develop on the surface of infected erythrocytes as the parasite matures are necessary for cytoadherence and are the points of attachment to endothelial cells in vivo (*7*) and to endothelial cells (*5*), melanoma cells (*6*), and monocytes (*3*) in vitro. An 88-kD leukocyte differentiation antigen (CD36), present on endothelial cells, melanoma cells, monocytes, and platelets and recognized by monoclonal antibody (MAb) OKM5, was shown indirectly to have a role in cytoadherence of malaria-infected erythrocytes (*8–10*). Malaria-infected cells can bind to all these cell types but not to cells lacking CD36. This glycoprotein was purified from platelets, where it is known as glycoprotein IV (GPIV) (*11*). We now present direct evidence that CD36 is a receptor for malaria-infected erythrocytes.

When a suspension containing 6% malaria-infected erythrocytes and 94% uninfected erythrocytes was incubated with immobilized CD36, selective binding of infected erythrocytes to CD36 is observed (Fig. 1). Binding to CD36 was observed with human and *Aotus* monkey erythrocytes infected with knobby (K+) *P. falciparum* isolates

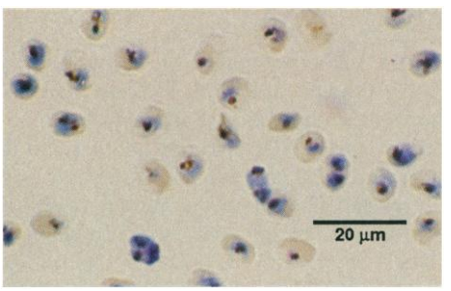

Fig. 1. Binding of *P. falciparum*–infected erythrocytes to CD36. Human erythrocytes infected with the K+ Brazilian ItG strain of *P. falciparum* (*12*) were cultured to the mature trophozoite stage of parasite development. Infected erythrocytes (2% suspension, 6% parasitemia) were washed, resuspended in RPMI 1640 medium, and a 2-ml portion was added to a plastic petri dish containing immobilized CD36 (*21*). After incubation at 37°C for 60 min, the dish was rinsed with RPMI 1640 to remove unattached erythrocytes, fixed with 2% glutaraldehyde, and stained with 1% Giemsa.

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capable of inducing knobs (Fig. 2). K+-infected erythrocytes did not bind to the unrelated platelet glycoprotein GPIb. Erythrocytes that are unable to adhere to host cells, including uninfected erythrocytes, erythrocytes infected with a knobless (K−) clone of the Palo Alto strain of *P. falciparum* that is unable to induce knobs (*12*), or erythrocytes infected with immature ring-stage parasites, did not bind to CD36.

^{125}I-labeled CD36 exhibited saturable binding to both K+-infected human erythrocytes and K+-infected *Aotus* monkey erythrocytes (Fig. 3). This binding was specific (>70% inhibited by a 100-fold excess of unlabeled CD36). Binding was also inhibited by MAb OKM5 (>80% inhibition by 10 μg/ml) and by rabbit antiserum to CD36 purified from platelets [>70% inhibition by 90 μg immunoglobulin G (IgG) per ml]. There was no binding of ^{125}I-GPIb to infected erythrocytes (Fig. 3A) and no binding of ^{125}I-CD36 to uninfected erythrocytes or to erythrocytes infected with a K− clone of *P. falciparum* (Fig. 3B). ^{125}I-labeled CD36 that had bound to infected erythrocytes migrated on SDS–polyacrylamide gel electrophoresis (SDS-PAGE) with an apparent molecular mass of 88 kD (Fig. 3D).

Cytoadherence is abolished by treatment of infected erythrocytes with low concentrations (10 μg/ml) of trypsin (*13*). Treatment of infected erythrocytes with this concentration of trypsin abolished ^{125}I-labeled CD36 binding (Fig. 3C). Soluble CD36 inhibited binding of infected erythrocytes to cultured endothelial cells and C32 melanoma cells (Table 1) and inhibited binding of ^{125}I-labeled MAb OKM5 to C32 melanoma cells (*14*). Addition of soluble CD36 to infected erythrocytes bound to melanoma cells reversed cytoadherence by 50 and 70% with 2.5 and 5.0 μg of CD36 per milliliter, respectively (*15*).

The surface glycoproteins recognized by MAb OKM5 on endothelial cells, melanoma cells, and monocytes appear to be identical or closely related to one another and to platelet GPIV, and we use the term CD36 to refer to all of them. All migrate as diffuse bands with an apparent molecular mass of 88 kD on SDS-PAGE (*8, 11, 14, 16*), and all are recognized in immunoblots by an MAb to CD36 purified from platelets (*14*) and by immunoprecipitation with polyclonal antibodies to CD36 purified from platelets (*17*). OKM5 interacts with platelets to induce aggregation and granule release and interacts with monocytes to induce production of reactive oxygen intermediates, mimicking the effects induced when infected erythrocytes bind to monocytes or platelets in vitro (*3*). These cellular responses suggest that MAb OKM5 and the infected erythrocyte

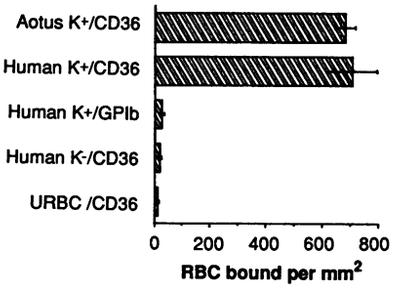

Fig. 2. Binding of *P. falciparum*–infected erythrocytes to CD36. *Aotus* erythrocytes infected with the K+ Malayan Camp strain (*22*) of *P. falciparum* (18% parasitemia), human erythrocytes infected with the K+ Brazilian ItG strain of *P. falciparum* (4% to 9% parasitemia), or human erythrocytes infected with the K− Palo Alto strain (*12*) of *P. falciparum* (6% parasitemia), were cultured to the mature trophozoite stage of parasite development. They were then washed, reacted with immobilized CD36 or GPIb (*21*), and processed as in Fig. 1. Results are expressed as the mean ± SD (n = 3 to 9). RBC, erythrocytes; URBC, uninfected erythrocytes.

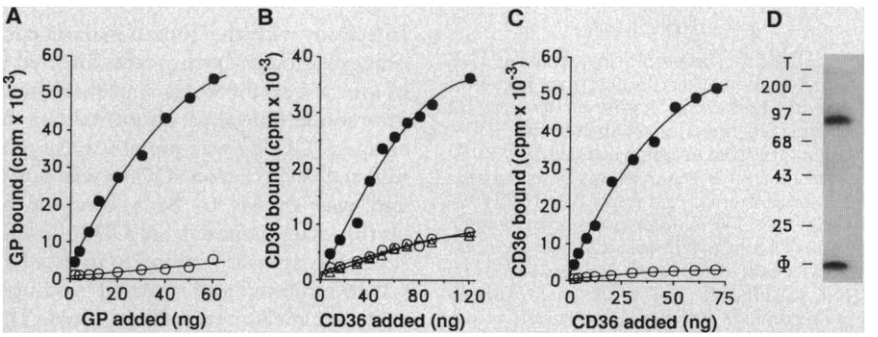

Fig. 3. Binding of ^{125}I-labeled CD36 to *P. falciparum*–infected erythrocytes. Portions (100 μl) of increasing concentrations of ^{125}I-labeled CD36 or GPIb (*23*) were added to wells of a round-bottom microtiter plate containing 60 μl of a 1.7% suspension of infected erythrocytes. After incubation for 1 hour at 37°C (A) or 4°C (B and C), cells were washed three times by centrifugation and resuspended in 200 μl of RPMI 1640 containing 0.1% bovine serum albumin, and total bound radioactivity was measured in a gamma counter. Results are plotted as the average of duplicate samples. (**A**) ^{125}I-CD36 (●) or ^{125}I-GPIb (○) was added to human erythrocytes infected with the K+ Brazilian ItG strain of *P. falciparum* (24% parasitemia). (**B**) ^{125}I-CD36 was added to uninfected erythrocytes (△, URBC), to human erythrocytes infected with the K+ (●) Brazilian ItG strain of *P. falciparum* (7% parasitemia), or to human erythrocytes infected with the K− (○) Malayan Camp strain of *P. falciparum* (10% parasitemia). (**C**) ^{125}I-CD36 was added to *Aotus* erythrocytes infected with the K+ (●) Malayan Camp strain of *P. falciparum* (19% parasitemia) or to infected erythrocytes previously treated with trypsin (○, Sigma) (10 μg/ml) at room temperature for 10 min followed by addition of soybean trypsin inhibitor (Sigma) (100 μg/ml final concentration). (**D**) Human erythrocytes infected with the K+ Brazilian ItG strain of *P. falciparum* (5% parasitemia) were incubated with 75 ng of ^{125}I-labeled CD36, extracted with sample buffer, resolved by 10% nonreducing SDS-PAGE, and subjected to autoradiography. The positions of prestained molecular weight markers (BRL, Bethesda, Maryland) are indicated on the left. T, top of the resolving gel; Φ, bromphenol blue dye front.

Table 1. Inhibition by soluble CD36 of *P. falciparum*–infected erythrocyte cytoadherence to melanoma cells and endothelial cells (*20*). C32 melanoma cells (American Type Culture Collection) were cultured (7.5×10^4 per well) in RPMI 1640 supplemented with 10% fetal bovine serum. Infected erythrocytes were cultured to the mature trophozoite and schizont stages of parasite development. Parasitemia for individual experiments ranged from 4 to 11% for ItG strain parasites in human erythrocytes and 15 to 29% for Camp strain parasites in *Aotus* erythrocytes. Portions (250 μl) of a 2% suspension of *P. falciparum*–infected erythrocytes in RPMI 1640 were incubated at 37°C with or without soluble CD36 for 60 min and then added to endothelial or melanoma cells in 24-well tissue culture plates. After 90 min at 37°C, unattached erythrocytes were removed by washing three times with RPMI 1640. The cells were then fixed with 2% glutaraldehyde and stained with 1% Giemsa. Quantitation of bound infected erythrocytes (IRBC) was calculated as the number of IRBC per 100 melanoma cells, or the total number of IRBC per 25 fields (magnification ×63) for confluent endothelial cells. Control values ranged from 784 to 2421 IRBC per 100 melanoma cells and 304 to 2272 IRBC per 25 fields of endothelial cells. Results are expressed as the mean percent inhibition, calculated as 100 × [1-(IRBC bound in presence of CD36/IRBC bound in absence of CD36)] ± SD, with the number of replicates in parentheses. NT, not tested.

CD36 (μg/ml)	Inhibition of binding (%)			
	Melanoma cells		Endothelial cells	
	Human/ItG	*Aotus*/Camp	Human/ItG	*Aotus*/Camp
1.25	18 ± 17 (7)	44 ± 3 (4)	41 ± 6 (2)	NT
2.5	43 ± 13 (9)	55 ± 10 (4)	58 ± 6 (6)	79 ± 1.4 (2)
5.0	80 ± 7 (6)	60 ± 7 (2)	71 ± 3 (2)	94 ± 0.4 (2)
10.0	81 ± 20 (8)	71 ± 5 (4)	NT	97 ± 0.8 (2)

surface ligand recognize an identical or closely related receptor binding site.

Thrombospondin (TSP), an adhesive glycoprotein, has also been proposed as a receptor for cytoadherence of infected erythrocytes (*18*). Our purified CD36 was not contaminated with TSP, as determined by Coomassie blue and silver staining of protein separated by SDS-PAGE and by enzyme immunoassay with antiserum to TSP (*17*). In addition, TSP is synthesized by many cells that do not have the capacity to bind *P. falciparum*–infected cells (*9*). This lack of correlation between the presence of TSP and binding argues against the specificity of TSP as a cytoadherence receptor. Surface expression of a receptor recognized by MAb OKM5 is correlated with the ability of various monocyte and melanoma cell lines to bind infected erythrocytes (*9, 10*). Furthermore, much higher concentrations of TSP than of CD36 are required for significant inhibition of cytoadherence to C32 melanoma cells (200 μg/ml versus <10 μg/ml), and neither TSP nor antibodies to TSP have been reported to reverse cytoadherence. It is possible that CD36 and TSP have sequence similarities that account for the binding of infected erythrocytes to either of these purified proteins when immobilized on plastic. Alternatively, CD36 and TSP may bind to independent ligands on the surface of K+-infected erythrocytes.

Although these results strongly suggest that CD36 is a malaria sequestration receptor, the identity of the complementary ligand on the infected erythrocyte surface is still unknown. It has been suggested that the cytoadherence ligand is a poorly immunogenic conserved domain that is masked by a more immunogenic, strain-specific moiety on the surface of *P. falciparum*–infected erythrocytes (*19*). The availability of purified CD36 should allow identification and characterization of epitopes on the cytoadherence ligand that may be useful for a malaria vaccine. Soluble CD36, or a fragment thereof that can reverse cytoadherence in vitro, may also be useful therapeutically for rapid reversal of sequestration in cerebral malaria or other complicated forms of this disease.

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16 September 1988; accepted 17 January 1989

The Mutations in Ashkenazi Jews with Adult G_{M2} Gangliosidosis, the Adult Form of Tay-Sachs Disease

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The adult form of Tay-Sachs disease, adult G_{M2} gangliosidosis, is an autosomal recessive disorder that results from mutations in the α chain of β-hexosaminidase A. This disorder, like infantile Tay-Sachs disease, is more frequent in the Ashkenazi Jewish population. A point mutation in the α-chain gene was identified that results in the substitution of Gly^{269} with Ser in eight Ashkenazi adult G_{M2} gangliosidosis patients from five different families. This amino acid substitution was shown to depress drastically the catalytic activity of the α chain after expression in COS-1 cells. All of these patients proved to be compound heterozygotes of the allele with the Gly to Ser change and one of the two Ashkenazi infantile Tay-Sachs alleles. These findings will aid in the diagnosis and understanding of β-hexosaminidase A deficiency disorders.

BOTH ADULT G_{M2} gangliosidosis and the infantile form of Tay-Sachs disease are autosomal recessive diseases caused by a deficiency of β-hexosaminidase A, a lysosomal enzyme composed of α and β chains (*1*). The enzyme deficiency is a consequence of mutations in the α-chain gene. As a result, there is a progressive accumulation of G_{M2} ganglioside, the natural substrate of β-hexosaminidase A, leading to degenerative changes in the nervous system. However, with respect to onset and severity, the two disorders are very different. In infantile Tay-Sachs disease, the age of onset is within the first year of life with death ensuing in early childhood after progressive motor and mental deterioration. In the adult disease (*2, 3*), onset usually occurs in the second or third decade with lower motor neuron, pyramidal tract, and cerebellar deterioration. In many cases psychosis seems to be an integral part of the disease and precedes the other neurological manifestations (*3*). Both the adult and infantile diseases are more prevalent in Ashkenazi Jews than in the general population, with the infantile form predominating.

Recently it has been shown that two different mutations in the α-chain gene un-

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