INFECTION AND IMMUNITY, May 1993, p. 2229-2232
0019-9567/93/052229-04$02.00/0
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Vol. 61, No. 5

Antigenic and Functional Differences in Adhesion of *Plasmodium falciparum*-Infected Erythrocytes to Human and Bovine CD36

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Cytoadherence by *Plasmodium falciparum*-infected erythrocytes (PRBC) to microvascular endothelium is, in part, mediated by the specific interaction between a parasite-derived erythrocyte surface ligand and a specific binding site on human CD36. We describe the selection for increased adhesion of PRBC to bovine CD36 and demonstrate that the molecular interaction between PRBC and bovine CD36 is independent of and distinct from the OKM5/8 monoclonal antibody epitopes which block PRBC-human CD36 binding.

Erythrocytes (RBC) parasitized with the lethal human malaria parasite, *Plasmodium falciparum*, sequester within microvascular capillary and postcapillary venular beds in such organs as the brain, heart, lungs, placenta, and intestine by binding to endothelial cells of the vessel walls. The adhesion of parasitized erythrocytes (PRBC) to the endothelium appears to be critical for the development of pathological complications of the disease and is mediated by multiple receptor-counter-receptor interactions. The evidence that one such receptor, CD36, is the dominant endothelial cell receptor for PRBC is compelling: (i) surface expression of CD36 (also termed GPIV or GPIIIb) on human platelets, monocytes, endothelial cells, and several tumor cell lines correlates with PRBC adhesion (4, 13, 18), while cells deficient in CD36 expression such as large-vessel endothelium (21), Nak^{a-} platelets (24, 26), and mutant tumor cell lines do not bind PRBC (13, 14); (ii) CD36 purified from human platelets or C32 melanoma cells and COS cells transfected with CD36 cDNA bind RBC infected with all laboratory-adapted and wild-type parasite isolates examined to date (3, 14, 16, 17); (iii) PRBC binding to cells is inhibited (>90%) by anti-CD36 monoclonal antibodies (MAbs) OKM5 and OKM8 (3, 4, 13, 14, 16); and (iv) anti-idiotypic antibodies against MAb OKM8 bind PRBC and immunoprecipitate a high-molecular-weight RBC membrane antigen of parasite origin, sequestrin (15).

The functional role of CD36 in hemostasis is less well understood. Platelet CD36 serves as a primary collagen receptor in the initial phase of platelet adhesion to collagen fibrils (22, 24). Also, CD36 functions as one of several binding proteins for the extracellular matrix glycoprotein thrombospondin (2). Human CD36 appears to be identical to PAS-IV (9, 10), an integral membrane protein of mammary epithelial cells, and belongs to a family of structurally related molecules which include LIMP II (25), a rat lysosomal membrane protein. Bovine CD36 has been purified from heart muscle microvascular endothelium and mammary epithelium and demonstrates immunological cross-reactivity and functional similarities to human CD36 (9, 10). Amino acid sequences of proteolytically cleaved fragments covering 60% of the bovine CD36 protein reveal >92% homology in primary sequence but significant differences in posttranslational glycosylation compared with human CD36 (7a, 12). The differences in electrophoretic mobility among human CD36 (88 kDa), bovine milk CD36 (78 kDa), and bovine heart endothelial cell CD36 (85 kDa) represent cell type-specific glycosylation since endoglycosidase F treatment reduced both human and bovine proteins to an identical apparent M_r of 57,000 (10).

Studies examining the functional and immunological cross-reactivity between human and bovine forms of CD36 report conflicting results. In a study by Catimel et al. (6), human platelet-derived CD36 and bovine mammary epithelial CD36 reacted equally to MAbs OKM5 and OKM8 while differing in adhesion to PRBC. In contrast, identical preparations of bovine mammary epithelial or bovine heart endothelial CD36 did not react with MAbs specific for human CD36 (OKM5, OKM8, and 8A6) in other studies (9). Furthermore, the lack of PRBC binding to a bovine CD36 which contains epitopes recognized by MAb OKM5 or OKM8 (6) contradicts several earlier studies in which the presence of the OKM5 and OKM8 epitopes correlated 100% with PRBC adhesion to purified human CD36 or cells expressing CD36 on their surface (3, 4, 13, 14, 17, 18). In the present study, we have reexamined the functional and antigenic similarities and differences between human and bovine CD36. We extend our earlier findings and confirm that human and bovine CD36 receptors are immunologically related yet possess distinct epitopes for MAb binding and specific binding-site structures for PRBC adhesion. The results of these studies are important for the development of effective inhibitors of PRBC-endothelial interactions based on the identification of PRBC binding sites on CD36.

Platelet-derived human CD36 and bovine milk mammary epithelial cell CD36 were purified as described previously (10, 23), adsorbed to 96-well microtiter plates, and assayed for reactivity with a panel of human CD36 MAbs by an indirect radioimmunoassay (Fig. 1). MAbs OKM5, OKM8 (Ortho Diagnostics, Raritan, N.J.), and NL07 (1) recognized human CD36-specific epitopes and did not bind bovine CD36, similar to observations by Greenwalt et al. (9). These MAbs recognize conformational epitopes (i.e., they do not bind to sodium dodecyl sulfate [SDS]-denatured CD36)

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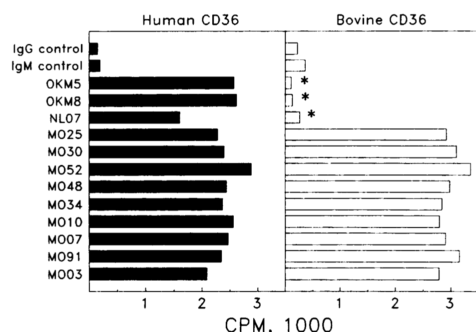

FIG. 1. Cross-reactivity of anti-human CD36 MAbs to human CD36 and bovine CD36. Proteins (1 μg/ml) were adsorbed onto 96-well microtiter plates and assayed for reactivity to a panel of anti-human CD36 MAbs (25 μg/ml). Reactivity was detected in an indirect radioimmunoassay by the addition of ^{125}I-labelled goat anti-mouse immunoglobulin (100,000 cpm). Results represent the mean of duplicate samples. Similar results were obtained on several different preparations of human and bovine CD36 proteins. Asterisks identify antibodies which differed significantly in their reactivity with human and bovine CD36. IgG, immunoglobulin G; IgM, immunoglobulin M.

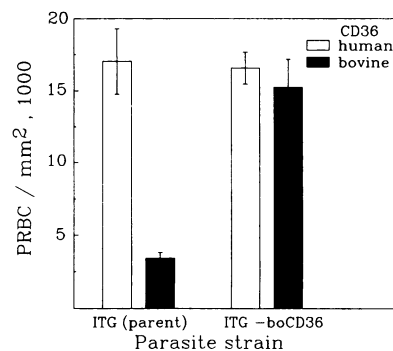

FIG. 2. Binding of PRBC to human CD36 and bovine CD36. ITG strain PRBC or PRBC selected by sequential panning on bovine CD36 (ITG-boCD36) were assayed for binding to plates coated with equivalent amounts of CD36 (1 μg/ml). Results represent the mean PRBC bound ± standard deviation of triplicate samples.

expressed on the cell surface and inhibit PRBC binding (3, 4, 13, 14, 16). In contrast, MAbs MO25, MO30, MO52, MO48, MO34, MO10, MO07, MO91, and MO03 raised against SDS-denatured human platelet-derived CD36 manifest cross-reactivity with human and bovine forms of CD36 by reacting with epitopes exposed on both the native and the denatured CD36 molecule.

In parallel experiments, PRBC binding to human CD36 and bovine mammary epithelial CD36 was assayed. Purified proteins (1 μg/ml) were adsorbed to culture dishes (Falcon 1007) overnight at 4°C. Nonspecific binding was reduced by blocking with phosphate-buffered saline–1% bovine serum albumin for 1 h. PRBC (ItG strain; 50% parasitemia, 2.5% hematocrit) selected for increased binding to human CD36 by serial panning (16) were added to the protein-coated plates for 1 h at room temperature. Plates were rinsed with RPMI 1640 to remove unattached RBC, and the bound RBC were subsequently fixed, stained with Giemsa, and examined for bound PRBC. These PRBC bound to human CD36 as expected. However, a low but significant number of PRBC were observed bound to bovine mammary epithelial cell CD36 (Fig. 2). Since PRBC binding to bovine CD36 has not been observed previously, a subpopulation of PRBC which bound to bovine mammary epithelial CD36 was selected by sequential panning on purified bovine CD36. These parasites were expanded, and RBC infected with these parasites were assayed for binding to human and bovine CD36. An increased binding (4.5-fold) to bovine CD36 was observed compared with PRBC selected on human CD36 alone (Fig. 2). Infected RBC adhesion to bovine CD36 is probably strain dependent since binding of RBC infected with two additional laboratory-adapted parasite isolates to bovine CD36 was reported to be undetectable (6, 9).

Curiously, PRBC binding to bovine CD36 appeared to be independent of the epitopes recognized by MAbs OKM5 and OKM8 since these MAbs do not recognize bovine CD36 by radioimmunoassay (Fig. 1) or enzyme-linked immunosorbent assay (ELISA) (Table 1), consistent with previously published observations (9). Indeed, preincubation of bovine CD36 with concentrations of MAb OKM8 which produced >87% inhibition of binding to human CD36 had no effect on PRBC binding to bovine CD36 (Table 1). Likewise, monospecific rabbit polyclonal human CD36 antisera which recognized both human and bovine CD36 by ELISA inhibited PRBC binding to human CD36 alone (Table 1). This implies that noninhibitory cross-reactive antibodies common to epitopes of both human and bovine CD36 make up the majority of the antibody specificities within the polyclonal antisera and that blocking antibodies are restricted to recognition of PRBC binding sites on human CD36 alone. In contrast, polyclonal anti-bovine CD36 inhibited PRBC binding to bovine CD36 but not to human CD36 (Table 1). MAb E-1 (8), a bovine CD36-specific MAb, did not inhibit PRBC binding to human or bovine CD36.

Two primary findings in this study may have significant implications for the design of effective inhibitors of PRBC-endothelium interactions. First, we have confirmed the findings of Greenwalt et al. (9) that human and bovine forms of CD36 possess unique species-specific epitopes and that the human CD36 epitope defined by MAbs OKM5 and OKM8 is part of the PRBC binding site as measured by antibody inhibition and anti-idiotype antibody mimicry of a CD36 binding domain (15). Epitopes common to human and

TABLE 1. Adhesion of PRBC and immunological reactivity to human and bovine CD36[a]

Antibody	Human CD36		Bovine CD36	
	% Inhibition	Optical density	% Inhibition	Optical density
OKM8	87 ± 4	1.09 ± 0.27	8 ± 6	0.09 ± 0.01
Rabbit anti-human CD36	97 ± 1	2.15 ± 0.12	13 ± 6	2.85 ± 0.03
E-1	−4 ± 16	0.11 ± 0.01	11 ± 11	2.55 ± 0.01
Rabbit anti-bovine CD36	3.6 ± 19	0.15 ± 0.004	67 ± 8	2.05 ± 0.05

[a] PRBC selected by serial panning to bind bovine CD36 were assayed for binding to both human CD36 and bovine CD36 in the presence or absence of MAb OKM8 (25 μg/ml), anti-bovine CD36 MAb E-1 (25 μg/ml), and affinity-purified polyclonal antibodies to human CD36 and bovine CD36 (125 μg/ml). Control PRBC binding to human CD36 and that to bovine CD36 were 13,066 ± 1,172 and 14,733 ± 1,258 PRBC bound/mm^2, respectively. Results represent the mean ± standard deviation of three samples. Antibody binding to proteins adsorbed onto 96-well microtiter plates was assayed by an indirect ELISA. Results represent the mean optical density ± standard deviation of triplicate samples.

bovine CD36 are recognized by several anti-human CD36 MAbs, confirming that these receptor molecules share significant homology in primary structure. Second, these results indicate that infected RBC of particular strains can bind bovine CD36 and that this binding is independent of the OKM5/8 epitope. Since the OKM5/8 epitope is destroyed by the ionic detergent SDS, the epitope and part or all of the PRBC binding site may be composed of discontinuous amino acid sequences held together in a tertiary structure by noncovalent forces or disulfide bonds. Short peptides based on the CD36 sequence, therefore, may not be potent inhibitors of PRBC-CD36 interaction. PRBC selected in vitro to bind both human and bovine CD36 should facilitate analysis of the binding-site structure through the production of constructs for chimeric proteins containing both bovine and human domains. Since the binding sites on human CD36 for PRBC and MAbs OKM5 and OKM8 overlap, it will also be interesting to examine PRBC binding to human CD36 mutagenized to eliminate the OKM5/8 epitope. The results of structure-function analysis of binding sites on human as well as bovine CD36 should have a substantial impact on the design of effective peptide or recombinant protein inhibitors of PRBC binding to CD36.

Evidence suggests that sequestrin may be identical to the previously described PfEMP1 antigen (11), which has been shown recently to exhibit considerable antigenic variability (5). PfEMP1 protein products from different parasite clones were shown to vary by several tens of thousands of daltons. Interestingly, a genomic clone of the 11-1 gene of *P. falciparum* has recently been shown to contain a large number of variable repeats (19). The antigenic variability of the sequestrin/PfEMP1 protein, the 11-1 gene product, and other *P. falciparum* erythrocytic-stage proteins (7, 20) may constitute a mechanism of immune evasion by the parasite and complicate attempts to produce a vaccine based on erythrocytic-stage cell surface antigens. Whether or not the size of sequestrin in PRBC selected to bind bovine CD36 differs from sequestrin in PRBC that bind only human CD36 is under investigation.

Our results are difficult to reconcile with those of Catimel et al. (6), who described MAb OKM5/8 binding to bovine mammary epithelial cell CD36. It is extremely unlikely that these differences are due to different preparations of bovine CD36 since this protein was from a common source. Furthermore, in our hands, bovine CD36 purified by three different preparative methods did not react with MAb OKM5, OKM8, or NL07. It is equally surprising that the preparations of bovine mammary epithelial cell CD36 used by Catimel et al. (6) which were reported to express the OKM5/8 epitope did not bind PRBC. In all studies reported previously, the presence of the OKM5 and OKM8 epitopes on purified CD36 or CD36 expressed on the surfaces of multiple cell types correlated with PRBC binding of all laboratory-adapted or wild-type isolates (14). Resolution of these discrepancies must await elucidation of the entire amino acid sequence of bovine CD36 and epitope mapping with a panel of blocking MAbs.

This work was supported in part by Public Health Service grant HL40858.

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