Lateral association between different transmembrane glycoproteins can serve to modulate integrin function. Here we characterize a physical association between the integrins $\alpha_3 \beta_1$ and $\alpha_6 \beta_1$ and CD36 on the surface of melanoma cells and show that ectopic expression of CD36 by CD36-negative MV3 melanoma cells increases their haptotactic migration on extracellular matrix components. The association was demonstrated by co-immunoprecipitation, reimmunoprecipitation, and immunoblotting of surface-labeled cells lysed in Brij 96 detergent. Confocal microscopy illustrated the co-association of $\alpha_3$ and CD36 in cell membrane projections and ruffles. A requirement for the extracellular domain of CD36 in this association was shown by co-immunoprecipitation experiments using surface-labeled MV3 melanoma or COS-7 cells that had been transiently transfected with chimeric constructs between CD36 and intercellular adhesion molecule 1 (ICAM-1) or with a truncation mutant of CD36. CD36 is known to engage in signal transduction and to localize to membrane microdomains or rafts in several cell types. Toward a mechanistic explanation for the functional effects of CD36 expression, we demonstrate that in fractionated Triton X-100 lysates of the MV3 cells stably transfected with CD36, CD36 was greatly enriched with the detergent-insoluble fractions that represent plasma membrane rafts. Significantly, when these fractionated lysates were reprobed for endogenous $\beta_1$ integrin, it was found that a 4-fold increase in the proportion of the mature protein was contained within the detergent-insoluble fractions when extracted from the CD36-transfected cells compared with MV3 cells transfected with vector only. These results suggest that in melanoma cells CD36 expression may induce the sequestration of certain integrins into membrane microdomains and promote cell migration.

Integrins comprise a large family of $\alpha \beta$ heterodimeric transmembrane proteins that function as key receptors for cellular attachment to the extracellular matrix and to cellular ligands (1). Integrin function extends beyond cell adhesion and, integrin-mediated signaling influences a range of biological processes including cellular division and migration, differentiation, and apoptosis (reviewed in Refs. 1–5). Integrin function is complex and is regulated at many levels. The binding of ligand to integrins can initiate signaling (1, 2), with bidirectional "cross-talk" occurring between integrins and other pathways (e.g. Refs. 6 and 7), including the functional modulation of other integrin molecules (8–10). Much emphasis has also focused on the process of "inside-out" signaling, where intracellular events enacted through the relatively short cytoplasmic tail sequences of integrins have a profound influence on the extracellular function of the integrin (1). In addition to the molecular interactions occurring between cytoplasmic proteins and the cytoplasmic domains of integrins (reviewed in Refs. 11 and 12), emerging evidence indicates that a number of surface transmembrane glycoproteins can associate with integrins and modulate their cellular functions (12).

Several classes of cell surface glycoproteins have been documented to play a role in integrin-mediated events, the most widely studied being the integrin-associated protein (IAP$^1$ CD47) and the unrelated transmembrane 4 superfamily (TM4SF or tetraspanins). IAP associates with the $\alpha_6 \beta_1$ integrin (13) and influences integrin-mediated signal transduction, phagocytosis, cellular migration, and chemotaxis (14–17). In vascular smooth muscle cells IAP also associates with the $\alpha_6 \beta_1$ integrin and modulates the chemotactic response of these cells to soluble collagen (18). Numerous TM4SF members have been shown to associate with $\beta_1$ integrins and with other TM4SF members (19–27). For example, the TM4SF molecule CD63 has been shown to specifically associate with $\alpha_6 \beta_1$ and $\alpha_6 \beta_1$ integrins (21), and the CD63-$\alpha_6 \beta_1$ complex was found to be associated with phosphatidylinositol 4-kinase activity, suggesting a novel integrin-signaling pathway (28). Furthermore, transfection studies with CD63 in a melanoma cell line resulted in marked inhibition of cell motility, an effect probably mediated by its influence on $\beta_1$ integrin function (29). Similarly, another TM4SF member, CD9, also has been shown to associate with $\beta_1$ integrins. (30).
integrins and to be implicated in cellular signaling, adhesion, motility, and differentiation (19, 20, 22, 30–32). It is believed that both IAP and the TM4SF molecules complex with integrins via extracellular domains, since the Ig domain of IAP is essential for effects on αβ2 integrin function (33) and the β1 integrin association with CD63 occurs independently of the integrin α-subunit cytoplasmic tail (20). More recently, Yaunch et al. (34) demonstrated using chimeras that the CD151 (TM4SF/αb3) association required extracellular domains found in CD151 and the α-chain of the integrin. Furthermore, in K562 cells, in which the αβ2 integrin initiates cell adhesion under flow conditions, the extracellular domains of the integrin determine its localization to microvilli, where both IAP and TM4SF proteins were also found to be strongly concentrated (35). Given the ubiquitous expression of both IAP and TM4SF, this suggests that regulation of integrin localization and function by these accessory molecules may play a widespread role in cellular processes.

Other surface molecules have also been implicated in the modulation of integrin-mediated functions. The widely expressed CD98 molecule (initially described as an early T-cell activation antigen) was found to regulate β1 integrin activation in a genetic complementation assay (36), and a physical association between CD98 and certain β1 integrins has recently been described (cited in Ref. 36); in the case of CD98, however, the association with β1 integrins appears to occur between cytosolic domains of the two glycoproteins. The CD98 interaction with β1 integrins appears to modulate the activation of T-cells (38) as well as the adhesion of breast carcinoma cells (39). Other examples of integrin-associated molecules that may modify or regulate integrin function include the ErbB-2 onco-gene, a receptor tyrosine kinase associated with breast cancer progression in vivo. This interaction demonstrated to facilitate phosphatidylinositol 3-kinase-dependent in vitro cell invasion by erbB-2-transformed 3T3 cells (39). The cancer metastasis-promoting molecule, urokinase plasminogen activator receptor (uPAR) also associates with and regulates β1 and β3 integrin function (40).

Another candidate co-associated regulator of integrin-function is the transmembrane glycoprotein CD36. The archetype member of a small gene family, CD36 is unrelated to integrins or to currently known integrin-accessory molecules. CD36 expression is prominent on the surface of platelets, capillary endothelial cells, macrophages, and cultured cell lines from a variety of sources including melanoma (reviewed in Refs. 41 and 42). A study using chemical cross-linking demonstrated that CD36 is spatially associated with the α3β3 integrin on the surface of platelets (43), and although the functional consequence of this association is not known, it has been shown that platelet activation achieved by the addition of anti-CD36 antibodies involves signaling through this integrin (44, 45). It is not known whether CD36 directly associates with integrins on other cell types (12), but there are several lines of evidence to suggest that certain functions ascribed to CD36 directly involve or implicate involvement of integrins.

The phagocytic uptake of apoptotic cells by macrophage requires the coordinate function of CD36 and the αβ3 integrin, possibly with involvement of the extracellular matrix protein thrombospondin functioning as a molecular bridge (46). Additionally, both CD36 and the αβ3 integrin have been shown to be involved in the phagocytosis of shed photoreceptor rod outer segments by retinal pigment epithelial cells (47, 48). Interestingly, CD36 functions as an adhesive cellular receptor for both thrombospondin (49) and collagen (50), specificities that also overlap with integrins of both the αv and β1 integrin subfamilies (1, 11). Fluorescence-activated cell sorting of C32 melanoma cells into populations expressing either high or low levels of CD36 showed that high CD36 expression defined a more fibroblastoid cell phenotype, with this population demonstrating enhanced tumor growth and motility in vivo studies using nude mice (51). Additionally, transfection of CD36 into human umbilical vein endothelial cells resulted in aberrant morphological changes affecting in vitro tube formation (52).

Taken together, these data suggest that certain functions attributed to CD36 directly involve or implicate involvement of integrins, particularly phagocytosis, cell adhesion, and migration. How this accessory function is enacted is not known.

Possibly significant in this regard is the reported association of CD36 with plasma membrane microdomains or “rafts” enriched in cholesterol, sphingomyelin, and glycolipids. Rafts can be isolated biochemically as low density Triton X-100-insoluble membranes, and such isolates have been termed detergent-insoluble glycosphingolipid-enriched complexes (DIGs), also described as DRM, GEMs, TIM, TFF, and LTDI (reviewed in (53–55)). CD36 was found to be highly enriched in DIGs from a variety of cellular sources including lung endothelium (56), platelets (57), and transfected COS-7 cells (58). Raft domains have been implicated in a variety of cellular processes including signal transduction, membrane trafficking, cholesterol homeostasis, the regulation of apoptosis, and cell motility (53–55, 59, 60). The plasma membrane invaginations known as caveolae may represent a specialized structural form incorporating raft components contained within the structural coat protein caveolin (61). Early studies suggested that integrins were not compartmentalized to rafts/caveolae (56, 62), but subsequent reports have indicated that a proportion of β1 integrins may physically and functionally associate with caveolin. For example, caveolin-1 can function to link Fyn kinase with the α1 integrin subunit, this resulting in downstream signaling promoting cell cycling (63). Furthermore the GPI-linked uPAR, β1 integrins and caveolin-1-co-immunoprecipitate in a single complex, and this complex appeared necessary for integrin-mediated adhesion and signaling (40, 64). Interestingly some Src-family kinases, including Fyn, Lyn, and Yes known to associate with CD36 (65, 66) are also associated with DIGs (56, 57, 67), suggesting that CD36 signaling and perhaps other CD36-mediated functions may be enacted via raft domains.

The present study was initiated to examine further the reported effect of CD36 on melanoma cell morphology and growth (51). A melanoma cell line that did not express endogenous CD36 was identified, and permanent CD36 transfectants were isolated. No differences were found in the morphology or growth rates of the transfectants compared with the parent cell line, but the CD36-transfected cells displayed higher haptotactic migration on extracellular matrix substrates. The possibility that integrins might be implicated in this functional effect was investigated by seeking a co-association between CD36 and endogenous integrins on the melanoma cell surface. Co-immunoprecipitation studies revealed a physical association between CD36 and the integrins αβ3 and αβ1, and this association was also shown on C32 melanoma cells expressing endogenous CD36. We then used transient transfection of chimeric constructs of CD36 to demonstrate that the association between CD36 and integrins requires the extracellular domain of the CD36 molecule. Finally, this association may occur within raft domains/DIGs, since ectopic expression of CD36 increased the proportion of β1 integrin(s) found within this fraction.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Antibodies**—The MV3 melanoma cell line (a gift of Dr. G. N. F. van Muijen) was isolated from a lymph node metastasis as described previously (68). C32 and WM115 melanoma and COS-7 mon-
key kidney cell lines were obtained from the ATCC (Manassas, VA). All cells were routinely cultured in Dulbecco’s modified Eagle’s medium (CSL, Parkville, Australia) supplemented with 10% fetal bovine serum (CytoSystems, Castle Hill, Australia), respectively. The monoclonal antibodies (mAbs) 13 and 16 directed against the βα and ββ subunits (TS2/7, 44H6, and 4F10, respectively), and polyclonal (pAb) anti-CD36 (69) were generous gifts of Dr. Ken Yamada (National Institutes of Health, Bethesda, MD). mAbs 13C2 and 23C6, directed against the αβ subunit and the αββ complex, respectively, were gifts from Dr. M. Horton (Imperial Cancer Research Fund, St. Bartholomew’s Hospital, London). AK7 (anti-α2, integrin), V9M5 (anti-CD36), and affinity-purified polyclonal anti-ICAM-1 were gifts from Dr. M. C. Berndt (Baker Institute, Victoria, Australia). I4H4 anti-ICAM-1 and I4E8 anti-CD36 mAbs were provided by Dr. W. Boyd (Queensland Institute of Medical Research, Australia). Polyclonal anti-calreticulin (LAR-090) and FMC56 (anti-CD9 mAb) were gifts of Dr. S. Dedhar (University of Newcastle, Australia). Polyclonal anti-caveolin was purchased from Transduction Laboratories (Lexington, KY).

Construction of Expression Vectors and Cell Transfection—The human cDNAs for CD36 (a gift of Dr. A. W. Boyd) and ICAM-1 (gift of Dr. D. Simmons, University of Oxford, Oxford, UK) in the pCDM8 vector were subcloned to the high level eukaryotic expression vector pEF.BOS (71) using XhoI restriction endonuclease digestion. To substitute the carboxy-terminal cytoplasmic tail of CD36 with the ICAM-1 cytoplasmic tail (CD36/ICAM tail chimera) the pEF.BOS ICAM-1 plasmid was used as template for polymerase chain reaction amplification using oligonucleotides 5′-GGcTatgcttatgccacgcgtgga-3′ and 5′-cccegagctgcatcctcgcag-3′, which introduced an SpI restriction site to the 5′-end of the product (mismatched oligonucleotide bases are represented in uppercase type). Following digestion with SpI/HincII, the resulting fragment was then subcloned to an MluI/EcoRI restriction “vector” fragment of pEF.BOS.CD36, thereby inserting the 31-amino acid ICAM-1 cytoplasmic tail following CD36 cysteine residue 466. A chimera consisting of the amino terminus of ICAM-1 and the CD36 carboxy-terminal transmembrane region and cytoplasmic tail (ICAM/CD36) was prepared by digestion of pEF.BOS.CD36 and pEF.BOS ICAM-1 plasmid DNA with HincII/HincII and MseI restriction endonucleases. The MseI-coupled to CseI-activated vector construct was validated by automated sequencing analysis.

Immunoblotting—Cell adhesion to extracellular matrix proteins (collagen type I, fibronectin, or laminin; Sigma) was measured as described previously (72). For migration assays, the underside of replicate Transwell migration inserts (8-μm pore size, 6.5-mm diameter; Costar, Corning, NY) were coted for 60–90 min at 37 °C with the indicated matrix proteins (20 μg/ml) or with bovine serum albumin (BSA; 0.5% (w/v) in 200 μl of PBS. The wells were washed once prior migration buffer (serum-free medium supplemented with 1% (v/v) newborn calf serum) was added to the top chamber. Cells were detached using trypsin-EDTA (0.25% (w/v) and 5 mM, respectively) and washed three times in migration buffer buffer before seeding to the top of the filter (105 cells in 100 μl). After 2 h at 37 °C, adherent cells were detached using trypsin/EDTA from the upper and lower membrane surfaces and separately pooled with the supernatants collected from each respective chamber. Cells were counted on a CASY-1 Cell Analyser (Scharfe Systems GmbH, Reutlingen), and cell migration was calculated as the percentage of cells harvested from the underside of the filter divided by the total number of cells counted. All assays were performed on triplicate wells and repeated several times.

Cell Surface Labeling and Immunoprecipitation—A modified lactoperoxidase method was used for cell surface radiolabeling with Na125I (Australian Radioisotopes, Lucas Heights, Australia) as described previously (73). For iodination of cell surface antigens, cells were resuspended in 10 mM sodium tetraborate, pH 8.8, 150 mM NaCl containing 50 μg/ml biotinamidocaproate N-hydroxysuccinimide ester (Sigma) and incubated for 15 min at room temperature before quenching by the addition of NH4Cl to a 10 mM final concentration. After washing with PBS, 110μm-labeled cells were lysed for 60 min in 0.5% (w/v) Triton X-100 lysis buffer (1% polyoxymethylene 10 oleyl ether (Sigma) in 25 mM Hepes, pH 7.5, 150 mM NaCl, 5 mM MgCl2 supplemented with protease inhibitors (2 mM phenylmethylsulfonyl fluoride, 10 μg/ml soybean trypsin inhibitor, and 2.5 mM iodoacetamide (all from Sigma)). Insoluble material was then removed by centrifugation for 10 min at 14,000 × g. The lysates were precleared twice using protein A beads (Amersham Pharmacia Biotech) and/or rabbit anti-mouse immunoglobulin G (DAKO, Carpinteria, CA) coupled to CNBr-activated Sepharose beads (Amersham Pharmacia Biotech) in combination with human transferrin (Calbiochem) coupled beads for 1–2 h at 4 °C. Lysates were then incubated for 2 h (biotin) or overnight (125I) with either mAb combined with rabbit anti-mouse immunoglobulin-coupled beads, with pAb with protein A beads (Amersham Pharmacia Biotech) and/or rabbit anti-mouse immunoglobulin G (DAKO, Carpinteria, CA) coupled to CNBr-activated Sepharose beads (Amersham Pharmacia Biotech) in combination with human transferrin (Calbiochem) coupled beads for 1–2 h at 4 °C, respectively. The beads were then washed five times in 1× washing buffer, and eluted with SDS-PAGE. Gels containing radiolabeled samples were fixed, stained, and dried, and the labeled protein bands were visualized by autoradiography. Cells containing biotinylated samples were transferred to nitrocellulose, and the bands were visualized by blotting with streptavidin–biotin horse-radish peroxidase complexes (Amersham Pharmacia Biotech) as described below for immunoblotting.

Immunoblotting—Cells were lysed and immunoprecipitated as de-
scribed above. Samples were resolved by SDS-PAGE and transferred to nitrocellulose using 25 mM Tris, 192 mM glycine, 20% methanol transfer buffer. Nitrocellulose membranes were blocked in 5% skim milk in TBS (20 mM Tris, pH 7.5, 500 mM NaCl, 0.05% Tween 20) before incubation with specific antibodies in 1% skim milk TBS. Membranes were then incubated with horseradish peroxidase-conjugated goat anti-mouse or rabbit immunoglobulins (Bio-Rad), and horseradish peroxidase complexes were detected by the Renaissance chemiluminescence system (PerKinElmer Life Sciences).

**Immunofluorescent Cell Staining and Confocal Microscopy—**MV3 cells were harvested as described above and allowed to attach to glass coverslips overnight. Following fixation with 4% formaldehyde (w/v) in PBS for 20 min at room temperature, the coverslips were washed in 0.1% BSA, PBS (PBSA) and permeabilized with Triton X-100 (0.1% (v/v) in PBSA) for 10 min at room temperature. Antibodies (diluted in PBSA) were then added overnight at 4 °C, and the coverslips were washed four or five times in PBS before mounting in Mowiol 4–88 (0.1 g/ml; Calbiochem) dissolved in a mixture of 12 ml of Tris-HCl (pH 8.5), 6 ml of Citifluor AF1 (Citifluor Ltd., London, UK), and 6 ml of distilled water. Fluorescein isothiocyanate-conjugated anti-integrin mAbs together with an anti-CD36 (11H5) TAMRA conjugate (prepared according to the manufacturer's recommended protocol; Molecular Probes, Inc., Eugene, OR) were used for the two-color analysis. Cells were examined by laser-scanning confocal microscopy (Zeiss LSM510, Zeiss Microscopes, Welwyn, UK).

**Detergent-insoluble Glycosphingolipid-enriched Membrane Raft Domains—**DIGs were prepared by floatation in sucrose density gradients as described previously (58). Briefly, whole cell lysates solubilized in 1% Triton X-100 in MES-buffered saline (pH 6.5) were adjusted to 40% sucrose and applied under a discontinuous 5–30% sucrose gradient. Following ultracentrifugation, DIGs were found to separate as a low density band in the top of the gradient, and equal protein amounts of each were analyzed by SDS-PAGE and immunoblotting (protein concentrations were determined using the BCA assay; Pierce). Densitometric analysis was performed on nonsaturating exposures of immunoblot films. Images were captured using a UMAX powerlook II scanner, and densitometric quantitation was performed using the NIH Image software package version 1.61.1 (written by W. Rasband, National Institutes of Health). Enrichment in DIGs was calculated as the total antigen density in DIG-containing fractions divided by the total antigen density detected across the gradient (percentage of antigen in DIGs).

**RESULTS**

The Integrins α5β1 and α6β1 Associate with CD36 on the Surface of Melanoma Cells—In order to determine whether CD36 influenced the adhesion and migration of melanoma cells in a system amenable to detailed analysis, we identified a well characterized metastatic melanoma cell line (MV3; Ref. 68) that was deficient in CD36 expression and transfected this with CD36. Several bulk populations of cells stably transfected with CD36 were derived by G418 selection, and one (uncloned) population expressing levels of CD36 comparable with that endogenously expressed by C32 and WM115 melanoma cells was selected for further study (MV3.CD36, Fig. 1A). In contrast to the report of Wong et al. (51), who found that C32 melanoma cells expressing high levels of CD36-antigen displayed a more fibroblastoid appearance relative to low CD36-antigen-expressing cells, both control (pEF.BOS vector) and CD36-transfected MV3-melanoma cells appeared morphologically indistinguishable (Fig. 1B), and we could detect no difference in the growth rates of the two populations (data not shown). Adhesion assays measuring attachment to fibronectin, collagen, or laminin substrates showed no significant differences between the MV3.BOS and MV3.CD36 cells either measured under time- or matrix protein concentration-dependent conditions (data not shown). In contrast, in haptotactic migration assays carried out in Transwell chambers, the CD36-transfected cells were found to display significantly increased migration on fibronectin, laminin, and collagen substrates but not on the BSA substrate control (Fig. 1C). Wong et al. (51) did not measure cell migration in vitro, but the high CD36-expressing C32 cells were more metastatic in vivo, and this, together with a more fibroblastoid morphology, suggests that these cells would be more migratory than the low CD36-antigen expressing cells. We did not test this directly by cell sorting, but for the C32 mixed population the corresponding migration rates were 32.4 ± 3.3, 13.7 ± 11.9,
suggest that CD36 was physically associating with $\beta_1$ integrins in the transfected MV3 cells.

To determine whether this was the case in melanoma cells expressing endogenous CD36, the same immunoprecipitation experiments were carried out with lysates of surface radioiodinated C32 and WM115 melanoma cells. As before, CD36 antibodies co-precipitated labeled bands in the region of $\beta_1$ integrins, although in these assays no CD36 was observed in the $\beta_1$ integrin immunoprecipitate under these conditions (Fig. 2B). These results suggest that the putative $\beta_1$ integrin(s) association with CD36 is not restricted to transfected cells but is representative of melanoma cell lines.

To identify specific integrin(s) associated with CD36, reprecipitation assays were employed. Phenotyping of the MV3 cells by flow cytometry demonstrated that they expressed each of the $\alpha_1$ to $\alpha_6$ subunits known to associate with the $\beta_1$ integrin subunit (1, 11). By this analysis, the $\alpha_2$, $\alpha_4$, and $\alpha_6$ integrin subunits appeared to be most abundant with relatively lesser amounts of $\alpha_1$, $\alpha_3$, and $\alpha_5$ antigen (Fig. 3A). These cells have been reported not to express $\alpha_5\beta_2$ (73), and we confirmed these results, although the cells were found to express $\alpha_5\beta_1$ (data not shown). In addition, MV3 cells are known not to express $\alpha_6\beta_4$ integrins (74). Immunoprecipitation analysis of Brij 96 lysates from surface radioiodinated MV3-CD36 cells substantiated these findings, although in this case the $\alpha_2$ integrin in particular appeared to label or precipitate less well or to be less abundant. In this experiment, it was also noted that $\alpha_4$ and $\alpha_6$ integrin subunit antibodies precipitated a labeled band with the same migration as immunoprecipitated CD36 (Fig. 3B). This result suggested that $\alpha_2$ and $\alpha_6$ are the predominant $\beta_1$ integrins associating with CD36 in the transfected MV3 cells. To substantiate this notion, surface-labeled MV3-CD36 cells were lysed in Brij lysis buffer and CD36 immunoprecipitated with anti-CD36 antibodies directly coupled to Sepharose 4B beads. Bound proteins were eluted from the beads with brief acid treatment and neutralized, and aliquots of the eluate were reprecipitated with antibodies to the $\beta_1$ and $\alpha_2$ integrin subunits. The results (Fig. 3C) indicated that the major integrin to co-precipitate with CD36 was $\alpha_2\beta_1$, with tracemounts of $\alpha_6\beta_1$ also associating with CD36. In repeat experiments confirming these results, we found in addition by reprecipitation with antibodies to $\alpha_5$, $\beta_2$, and $\beta_5$, that none of these $\alpha_5$ integrins were associated with CD36 on the transfected MV3 cells (data not shown).

The co-association was then confirmed by immunoblotting (Fig. 3D). MV3-CD36 cells were lysed in Brij lysis buffer and immunoprecipitated for CD36 and for $\alpha_2$, $\alpha_4$, and $\alpha_6$ integrins. When the resulting immunoprecipitates were immunoblotted for CD36, both $\alpha_2$ and $\alpha_4$ lanes showed co-precipitated CD36, whereas $\alpha_6$ did not. Taken together, these results suggested that $\alpha_2\beta_1$ and $\alpha_6\beta_1$ form a physical association with CD36 on the surface of transfected MV3 cells. To ensure that the association also applied to endogenous CD36 on the surface of melanoma cells, the elution and reprecipitation experiment described above was repeated with C32 melanoma cells. These cells also express each of the integrins $\alpha_1$ to $\alpha_6$, although $\alpha_1$, $\alpha_2$, $\alpha_5$, and $\alpha_6$ are relatively less abundant by both flow cytometry and immunoprecipitation analyses (data not shown). With the C32 melanoma cells, reprecipitation of the integrins from the acid eluate obtained from the CD36 immunoprecipitate revealed associated $\alpha_2\beta_1$, but in this case not $\alpha_5\beta_1$ (Fig. 3E), but it is likely that the relatively weaker association with $\alpha_5\beta_1$ seen with the MV3 cells (Fig. 3C) would not be seen in precipitates from C32 cells in which the precipitated signal is much weaker.

To confirm that the association between the $\beta_1$ integrins and CD36 occurred in vivo and to identify the cellular location(s) of
this association, dual color immunofluorescence and confocal microscopic analysis were performed on the transfected MV3 cells after staining at the cell surface for α2 or α3 integrins and CD36. No specific association between α2 integrin and CD36 integrin was identified in these cells (data not shown). In contrast, there was strong co-localization between CD36 and α3 integrin in a high proportion of the cells examined, and the sites of association were identified as being predominantly on cell surface projections and/or membrane ruffles (Fig. 4).

Association with Integrins Requires the Extracellular Domain of CD36—To elucidate the nature of the CD36/β1 integrin association, chimeras between CD36 and ICAM-1 were utilized (Fig. 5A). These constructs were transiently transfected into the MV3 melanoma cells, and 3 days following transfection, the cells were surface-labeled with biotin, lysed in Brij 96 lysis buffer, and immunoprecipitated with antibodies directed against CD36, ICAM-1, and β1 integrins. The results demonstrate that, as was shown for the permanently transfected MV3.CD36 cells, immunoprecipitates of CD36 from MV3 cells transiently transfected with wild-type CD36 co-precipitated β1 integrins (Fig. 5B); note that using the biotinylation protocol, the α-subunit(s) of β1 integrins are less strongly labeled in comparison with radioiodination (compare with Fig. 3B). The substitution of the CD36 carboxyl-terminal cytoplasmic tail with that of ICAM-1 did not affect the β1 integrin association in the transfected cells (Fig. 5B; CD36/ICAM tail). Additionally, as demonstrated for wild-type CD36 above (Fig. 3D), immunoblotting of the immunoprecipitates of specific β1 integrins with antibodies against CD36 revealed that the CD36/ICAM tail chimera co-precipitated with α2 and α6 integrins but not with the α2 subunit (Fig. 3C), corroborating the results presented in Fig. 5B and further confirming the specificity of the CD36-β1 integrin interaction.

The MV3 cells were then transfected with an ICAM/CD36 chimera, encoding the extracellular domain of ICAM-1 and the CD36 carboxyl-terminal transmembrane/cytoplasmic sequences (Fig. 5A), and the cell lysates were precipitated with an anti-ICAM-1 mAb. This antibody was found to precipitate endogenous ICAM-1 from the control transfected MV3 cells (Fig. 5D, BOS), and no integrin bands were seen to co-precipitate with the ICAM-1 molecule. In cells transfected with the ICAM/CD36 construct, the chimeric molecule was found to co-precipitate with endogenous ICAM-1, but again there were no integrin bands identified in the precipitate (Fig. 5D). There-
fore, since no association was seen when the extracellular region of CD36 was replaced with ICAM-1, although the transmembrane domain and cytoplasmic tail of CD36 were left intact, these data suggest that the association between CD36 and integrin molecules occurs extracellularly.

These findings established that the association between the \( \beta_1 \) integrins and CD36 occurs in the absence of the carboxyl-terminal cytoplasmic tail of CD36. However, the predicted CD36 polypeptide contains 471 amino acids with hydrophobic regions resembling transmembrane domains present adjacent to both the amino- and carboxyl-terminal regions. The short amino-terminal hydrophobic region resembles an uncleaved signal peptide (75) with only the initiator methionine being absent in the mature protein (76). From these data, it has been proposed that CD36 is ditopic in an “inverted horseshoe” membrane configuration (41), although it has been controversial as to whether the amino-terminal transmembrane domain actually serves to anchor the protein (77–79). If the amino-terminal region does form a transmembrane domain, therefore, it is possible that an association between the \( \beta_1 \) integrins and CD36 could occur in this region, either in the amino-terminal cytoplasmic domain or in the transmembrane domain.

To investigate this, a molecule was constructed lacking the amino-terminal and cytoplasmic domains; this construct was tagged with a FLAG epitope and incorporates a cleavable signal sequence (FN\( \Delta \)TM.CD36; Fig. 6A). Upon transfection, however, this construct was very poorly expressed in the MV3 cells. Preliminary analyses revealed that reasonable levels of expression could be obtained with this construct in transiently transfected COS-7 cells (data not shown). Therefore, COS-7 cells were transfected with the control BOS vector, wild-type CD36, or the FN\( \Delta \)TM.CD36 construct, and the lysates of biotin-labeled cells were subjected to immunoprecipitation analysis. Immunoprecipitates of wild-type CD36 showed a specific co-precipitated band with identical mobility to the \( \beta_1 \) integrin subunit (Fig. 6B), indicating that the transfected human CD36 can associate with endogenous monkey \( \beta_1 \) integrin(s). Similarly, both the amino-terminal truncate of CD36 (FN\( \Delta \)TM.CD36; Fig. 6B) and the CD36/ICAM tail mutant (Fig. 5A and data not shown) also co-precipitated endogenous \( \beta_1 \) integrins from these cells. Taken together, these results establish that the association between \( \beta_1 \) integrins and CD36 requires the extracellular domain of CD36.

**Expressed CD36 Sequesters a Proportion of \( \beta_1 \) Integrin to DIGs in Transfected MV3 Melanoma Cells**—Membrane microdomains, or rafts, are specialized compartments of the plasma membrane that are highly enriched in cholesterol and glycosphingolipids (53–55, 80). They are also greatly enriched in many signal-transducing molecules and are therefore thought to represent signaling microdomains within the membrane (56, 57, 67). These rafts are relatively insoluble in detergents such as Triton X-100 by virtue of their lipid constituents, and this feature has enabled the well validated biochemical approach of DIG isolation to identify the proteins located within the microdomains (53, 56, 67, 80). Relatively few transmembrane glycoproteins have been identified as raft-associated in this way. However, CD36 has been characterized as one such molecule that has been shown to partition into DIGs isolated from murine lung endothelial cells (56), human platelets (57), and transfected COS-7 cells (58), where it may engage in signal transduction (44, 65, 81). We therefore determined whether CD36 also partitioned into DIGs isolated from the transfected MV3 cells, and, because this may provide a possible mechanism for the influence of CD36 on integrin function, we examined whether CD36 expression altered the proportion of \( \beta_1 \) integrin contained in DIGs.

MV3.BOS and MV3.CD36 transfected cells were lysed with 1% Triton X-100 in MES-buffered saline buffer and subjected to flotation on sucrose gradients as described under “Experimental Procedures.” Following fractionation of the gradient, equal protein amounts from each fraction were separated on SDS-PAGE, and the distribution of various antigens was analyzed.
β1 Integrins Associate with CD36

β Integrins Associate with CD36

Fig. 5. The association with β1 integrins does not involve the carboxyl-terminal transmembrane/cyttoplasmic domains of CD36. A, schematic representation of CD36, ICAM-1, and chimeric constructs. The hatched areas represent sequences derived from ICAM-1. TM indicates the location of the predicted transmembrane domains. The wild-type CD36, wild-type ICAM-1, CD36/ICAM tail, and ICAM/CD36 constructs encode 472, 532, 497, and 492 amino acids, respectively. B, MV3 cells (∼2 × 10^6 cells) were transiently transfected with the empty pEF.BOS vector (BOS), wild-type CD36, or the CD36/ICAM tail chimera (CTTail). Following transfection, the cells were biotin-labeled on the cell surface and lysed in 1% Brij lysis buffer, and immunoprecipitations (i.p.) were performed for CD36 (VM58, IA7, and IE8 mAb mixture) using 90% of the total cell lysate and for the indicated integrin subunits (αo, αo, and αo) before resolving the samples by SDS-PAGE under reducing conditions and transferred to nitrocellulose, and biotinylated proteins were visualized with streptavidin-horseradish peroxidase as described under “Experimental Procedures.” C, MV3 cells (∼10 × 10^6 cells) were transiently transfected with the CD36/ICAM tail construct before lysis with 1% Brij lysis buffer. Immunoprecipitations were performed for CD36 (11H5 mAb) and the indicated integrin subunits (αo, αo, and αo) using 10% of cell lysate. The samples were resolved by 7.5% SDS-PAGE under reducing conditions and transferred to nitrocellulose, and biotinylated proteins were visualized with streptavidin-horseradish peroxidase as described under “Experimental Procedures.” D, MV3 cells (∼2 × 10^6 cells) were transiently transfected with either CD36, BOS, or the ICAM/CD36 (I/CD36) chimera and analyzed as described for B. The lysates were subjected to immunoprecipitation with either the anti-CD36 mAb mixture (CD36; IA7/IE8), or with anti-ICAM-1 (BOS and I/CD36; IH4 mAb). Note that the increased migration seen with the expressed ICAM/CD36 construct results from differences in glycosylation. All results were confirmed in three separate experiments.

Fig. 6. The extracellular domain of CD36 is implicated in its association with β1 integrins. A, schematic representation of wild-type CD36 and the epitope-tagged amino-terminal truncation of CD36 (FNΔTM.CD36). TM, IL3, and F indicate the locations of the predicted transmembrane domains, IL3 signal sequence, and the FLAG epitope tag sequence, respectively. B, COS-7 (∼2 × 10^6 cells) were transiently transfected with either pEF.BOS (control), CD36, or the FNΔTM.CD36 construct and analyzed as described in the legend to Fig. 5. The lysates were subjected to immunoprecipitation (I.P.) either with the anti-CD36 mAb mixture (BOS and CD36; VM58/IA7/IE8 mAbs) or with anti-FLAG (FNΔTM, M2 mAb). Ten percent of the total lysate for each transfection was immunoprecipitated for β1 (pAb). The strong band denoted by the asterisk represents a nonspecific band precipitated with the M2 mAb. Results were confirmed in two separate experiments.

by immunoblotting. As expected, CD36 was not detected in the BOS-transfected cells, but, following CD36 transfection, it was found to be greatly enriched in the fractions representing the light-refractile DIG band (Fig. 7, compare A and B). Control and CD36-transfected lines displayed abundant caveolin, also concentrated in the DIG fractions. The same fractions were also probed for an ER resident protein, calreticulin (82), and, as expected, this did not co-migrate with the DIGs but was primarily confined to the fractions of the gradient corresponding to soluble proteins (Fig. 7, A and B). The β1 integrin subunit was observed predominantly in the soluble region of the gradient from both CD36 and control transfectants, the upper band representing the mature molecule and the lower band corresponding to the precursor (69). However, whereas in the control (MV3.BOS) transfectants a small amount of β1 integrin was found in DIGs, being entirely the mature form, the proportion of this mature protein found in the DIG fraction was considerably relatively enriched in fractions from the CD36 transfectants. CD9, a member of the TM4SF family and known to form a physical association with β1 integrins (20, 22, 23, 25, 32), was also examined for its distribution in these gradients. This molecule was well represented in most fractions but was most enriched in fractions 5 and 6 just below the DIGs and above the “soluble” region of the gradient. These fractions do not contain much biotinylated material from lysates of cells surface-labeled with biotin, and they are also enriched in the Golgi marker GS25; therefore, they may represent intracellular material. Notably, however, there appeared to be no difference in the relative amounts of CD9 in the DIG fractions from the control- or CD36-transfected cells. Densitometric analyses of the data confirmed these observations.

R. F. Thorne and G. F. Burns, unpublished data.
**β₁ Integrins Associate with CD36**

These results suggest that CD36 can sequester a proportion of β₁ integrin into membrane microdomains, where it is more likely to encounter signaling molecules that could modulate the function of the integrins. It is possible that substantially higher proportions of α₁β₁ and α₁β₃ were specifically sequestered, but blotting antibodies to the α-subunits were not available for this study.

**DISCUSSION**

It is increasingly being recognized that integrin function can be modulated by association with other transmembrane glycoproteins, as well as by cytosolic proteins, and several such integrin-associated glycoproteins have been identified. To this list can now be added CD36. As demonstrated here in melanoma cells, CD36 forms a physical association with the β₁ integrins α₁β₁ and α₁β₃, and this association requires the extracellular domain of CD36; co-expression of CD36 in MV3 melanoma cells results in increased migration on extracellular matrix components, indicating that the association has functional consequences. This requirement for the extracellular domain of CD36 for integrin association is shared with other molecules laterally associated with integrins, including IAP, the GPI-linked uPAR, and, notably, members of the TM4SF family (20, 24, 33–35, 40).

The specific integrins identified as associating with CD36 were α₁β₁ and, to a lesser extent, α₁β₃. It is striking that these particular integrins are also those most frequently identified as associating with members of the TM4SF family (21, 22, 25, 27, 28, 32). In order to demonstrate the association of CD36 and integrins by co-immunoprecipitation, we lysed the cells in Brij 96 detergent. Some co-precipitation (although less) was also demonstrated after cell lysis in 1% octyl glucoside, but the association was disrupted after lysis of the cells in 1% Nonidet P-40 or 1% Triton X-100. These features are shared with several reported TM4SF-integrin associations, and Hemler and associates (21, 22, 24, 25) have rigorously demonstrated that such associations are not the result of detergent artifact but rather depend upon the strength of the association and its capacity to withstand “stringent” detergents. Some suggested TM4SF-integrin associations have been based upon co-immunoprecipitation results after lysis in CHAPS, Brij 99, or Brij 58 but do not withstand lysis in Brij 96 (as used in the present study), which is more hydrophobic; therefore, the CD36-integrin association is reasonably robust. Recently, however, Yaunch et al. (26, 34) reported a direct association between an extracellular domain of the TM4SF protein CD151 and integrin α₁β₁ that occurred at high stoichiometry even after cell lysis in 1% Triton X-100 and was relatively resistant to denaturing detergents. Further, an association between CD151 and α₁β₃ integrin was found on every cell and tissue type examined. CD151 can also associate with other TM4SF proteins under milder conditions of lysis (26), leading these authors to postulate that some accounts of TM4SF-integrin association may be due to co-precipitation with CD151-α₁β₁ complexes. These findings suggest the possibility that CD36 may form part of a ternary complex with the integrin and CD151. Perhaps in support of this, in a chemical cross-linking study of platelets, Dorathy et al. (43) demonstrated that the platelet integrin α₁β₃ formed a physical association with both CD36 and the TM4SF protein, CD9. Studies are in progress to determine whether CD36 associates with CD151 in melanoma cells, but it should be noted that whereas CD151 associates with the immature α₆ precursor (34), CD36 was found to associate only with the mature β₁ molecule.

An important finding in the present study was that β₁ integrin contained within the DIG fraction isolated from MV3...
melanoma cells transfected with CD36 was relatively enriched compared to control cells. Several studies have validated the concept that such biochemically isolated DIGs are representative of specialized plasma membrane microdomains or rafts (53, 56, 67, 80). Caveolin, the marker coat protein of the distinct membrane structures known as caveolae was also found in this fraction, raising the possibility that association with CD36 facilitates the entry of $\beta_1$ integrins into caveolae. Caveolae are thought to bud from membrane microdomains in a process controlled by caveolin oligomers, and, in addition to their characteristic enrichment in cholesterol and sphingolipid, they are greatly enriched in cellular signaling molecules (56, 57, 67). Integrins are not generally considered to locate to caveolae (56, 62), but recent work has revealed that a fraction of $\beta_1$ integrin in Fisher rat thyroid cells associates with both caveolin and the protein-tyrosine kinase Fyn, and Fyn-dependent Src-mediated signaling in response to integrin ligation was found to be dependent upon the co-expression of caveolin-1 (63). Also, by use of antisense methodology in kidney 293 cells, Wei et al. (64) found that caveolin was important to $\beta_1$ integrin-dependent adhesion to fibronectin and the activation of focal adhesion kinase. The authors propose that $\beta_1$ integrins form a complex with caveolin that is stabilized by the nonintegrin, GPI-linked urokinase receptor, uPAR; this complex, in turn, regulates the ability of caveolin to regulate members of the Src family of protein-tyrosine kinases surrounding integrins.

Integrins co-precipitate with endogenous and transfected uPAR in a complex that also contains caveolin (40, 83). Caveolin itself can also bind cholesterol, Src family kinases, heterotrimeric G proteins, and Ha-Ras (84, 85), thereby contributing to the formation of caveolae rich in signaling molecules. It might be considered therefore that by associating with $\beta_1$ integrins through its external domain, CD36 can substitute for or mimic uPAR in providing a bridge for caveolin association. Three observations militate against this possibility. First, in our co-precipitation studies, we were unable to detect any caveolin in the CD36/β1 precipitates by immunoblotting. Second, in an immunofluorescence study of the CD36-transfected MV3 cells, it was found that the CD36 appeared to compete with caveolin such that cells expressing high levels of CD36 displayed considerably fewer of the flask-like invaginations representative of morphological caveolae. The reason for this is not known at present, but this result does suggest that CD36 is not simply a passive facilitator of caveolae association. Third, CD36 locates to biochemically isolated plasma membrane rafts even in the absence of caveolin; in platelets that contain a vitronectin binding site and can regulate the adhesive function of associated integrins include uPAR, which contains a vitronectin binding site and can regulate the relative binding of $\alpha_3\beta_1$ integrin to fibronectin or vitronectin (40), and the 67-kDa monomeric laminin receptor that regulates the laminin-binding function of the $\alpha_6\beta_1$ integrin (95). CD36 has been described as a cell surface receptor for both collagen and thrombospondin (49, 50). Tandon and colleagues (96, 97) have recorded that CD36 expression influences the rate of platelet attachment and spreading on tissue I collagen mediated by the $\alpha_IIb\beta_3$ integrin. $\alpha_IIb\beta_3$ integrin also can bind collagen; however, in our study with transfected MV3 melanoma cells we could detect no influence of CD36 expression on the degree or rate of spreading of these cells on collagen or on the $\alpha_IIIb\beta_3$ substrate, laminin (data not shown). CD36 binding to the pericellular matrix glycoprotein, thrombospondin, may be a more attractive candidate for the modulatory role of CD36 in cell migration. Thrombospondin is abundantly secreted by many migratory cell types, including melanoma cells; it can bind to several extracellular matrix proteins and, functioning as an antiadhesive molecule, can promote cell migration (reviewed in Ref. 98). Thrombospondin contains several distinct domains able to bind to discrete cell surface receptors, including CD36 and $\alpha_3$ and $\beta_1$ integrins (98), and fibroblast migration on thrombospondin has been shown to include a role for both integrins and CD36 (99). Of interest, the integrin-associated protein (iap or CD47), which has been demonstrated to physically associate with integrins and to modulate their function (13–16, 33), also functions as a receptor for the carboxyl-terminal region of thrombospondin (17, 100). In a recent study, Wang and Frazier (18) demonstrated that iAP physically associates with $\alpha_IIb\beta_3$ integrin on aortic smooth muscle cells and that thrombospondin peptides able to bind iAP stimulated the chemotaxis of those cells on gelatin-coated filters.

Precisely how CD36 might regulate integrin function remains to be determined, but several scenarios can be envisioned. By the recruitment of adjacent integrins into membrane microdomains together with Src-related kinases, CD36 could serve to promote integrin-mediated signaling. Further, membrane microdomains are highly enriched in gangliosides (80), which themselves can regulate protein kinase function (92) and, more particularly, have been shown physically to associate with Arg-Gly-Asp-directed integrins and to be involved in their adhesive function (72, 93, 94). Not necessarily unrelated to such a role in sequestration is the possibility that CD36 can function as an accessory molecule, complementing integrin ligation with certain extracellular matrix ligands. Precedents for integrin accessory molecules regulating or stabilizing the adhesive function of associated integrins include uPAR, which contains a vitronectin binding site and can regulate the relative binding of $\alpha_3\beta_1$ integrin to fibronectin or vitronectin (40), and the 67-kDa monomeric laminin receptor that regulates the laminin-binding function of the $\alpha_6\beta_1$ integrin (95).

Finally, at the initiation of this study, we expected to find an association between CD36 and $\alpha_3\beta_1$ integrin because of reports of the coordination of these receptors in the phagocytic uptake of apoptotic cells (46). However, we were unable to demonstrate co-precipitation of these molecules from WM115 melanoma cells that express both in abundance, or from MV3 cells transfected with both CD36 and $\beta_3$ integrin (43). The ready demonstration of a CD36-$\alpha_3\beta_1$ association under these conditions suggests that, if CD36 and $\alpha_3\beta_1$ integrin are coordinately in phagocytosis, they do so without being in physical association. However, while a role for CD36 in this function has been well validated in transfection studies (101) and a CD36 family member, croquemort, has been found in genetic studies to be essential for the removal of apoptotic cells during Drosophila development (102), similar studies have not been carried out to determine the role of $\alpha_3\beta_3$. Studies documenting a role for $\alpha_3\beta_3$ in this function have depended upon antibody-induced or peptide agonist-induced inhibition (46, 103–106). More recent work has established that the engagement of $\beta_3$ integrins with antibody or ligand can

---

3 R. G. Parton, R. F. Thorne, and G. F. Burns, unpublished data.
β1 Integrins Associate with CD36

In the result of down-regulating the function of β1 integrins in a signal-mediated process known as transendothelial inhibition (8, 9). Further, it has been established that αβ1 integrin participates in phagocytosis mediated by human breast cancer cells (107), most of which express CD36 (91). Therefore, the findings reported here of an association between CD36 and αβ1 integrin may indicate that the conclusions of studies implicating αβ1 in the phagocytic process may warrant further evaluation to assess the role of β1 integrins and to consider the possibility that αβ1 engages in integrin receptor cross-talk.

Acknowledgments—We thank all those investigators mentioned who supplied reagents essential to this work. We are grateful to Dr. Eric Brouillette (Division of Infectious Diseases, Washington University, St. Louis) for critically reviewing a draft version of the manuscript and for valuable suggestions.

REFERENCES

1. Hynes, R. O. (1992) Cell 69, 11–25
2. Julenius, E. L., Naumov, I. V., J. Cell Biol. 120, 577–585
3. Clark, E. A., and Brugge, J. S. (1995) Cell Adhesion Commun. 2, 1313–1322
4. Schwartz, M. A., Schaller, M. D., and Ginsberg, M. H. (1995) Annu. Rev. Cell Biol. 11, 549–590
5. Giancotti, F. G., and Ruoslahti, E. (1999) Science 283, 1042–1046
6. Monier-Gavelle, F., and Duband, J.-L. (1997) J. Cell Biol. 136, 1663–1681
7. Rainger, G. E., Buckley, C., Simmons, D. L., and Nash, G. B. (1997)Curr. Opin. Immunol. 9, 316–325
8. Blystone, S. D., Lindberg, F. P., Brown, E. J. (1994) J. Cell Biol. 127, 1129–1137
9. Dzau-Gonzalez, R., Forgy, J., Steinber, B., and Ginsberg, M. N. (1996) Mol. Biol. Cell 7, 1995–1998
10. Porter, J. C., and Hogg, N. (1997) J. Cell Biol. 6, 1437–1447
11. Dedhar, S. (1995) J. Cell Biol. 131, 1629–1635
12. Hemler, M. E. (1998) Curr. Opin. Cell Biol. 10, 374–382
13. Berditchevski, F., Bazzoni, G., and Hemler, M. E. (1995) J. Biol. Chem. 270, 13493–13500
14. Radford, K. J., Thorne, R. F., and Hersey, P. (1996) J. Cell Biol. 134, 297–305
15. Monier-Gavelle, F., and Duband, J.-L. (1997) J. Cell Biol. 136, 1663–1681
16. Giancotti, F. G., and Ruoslahti, E. (1999) Science 283, 1042–1046
17. Hudson, D. W., Pearce, F. A., Zhang, R., Silverstein, R. L., Frazier, W. A., and Bouch, N. P. (1997) J. Cell Biol. 138, 707–717
18. Brown, D. A., and London, E. (1997) Biochem. Biophys. Res. Commun. 240, 812–818
19. Ko, Y., Lee, J. S., Kang, Y. S., Ahn, J. H., and Seo, S. J. (1999) Immunol. 150, 1165–1170
20. Rubenstein, E., Le Naour, F., Billard, M., Prenant, M., and Boucheix, C. (1995) Eur. J. Immunol. 25, 1273–1281
21. Berditchevski, F., Zutter, M. M., and Hemler, M. E. (1996) Mol. Biol. Cell 7, 297–305
22. Falcioni, R. (2000) J. Cell Biol. 2785–2794
23. Radford, K. J., Thorne, R. F., and Hersey, P. (1996) J. Cell Biol. 134, 297–305
24. Mannion, B. A., Berditchevski, F., Kraeft, S.-K., Chen, L. B., and Hemler, M. E. (1996) J. Immunol. 157, 2039–2047
25. Tachibana, I., Bodorova, J., Berditchevski, F., Zutter, M. M., and Hemler, M. E. (1998) Mol. Biol. Cell 9, 865–874
26. Slupsky, J. R., Seehafer, J. G., Tang, S. C., MasSELLia-Smith, A., and Shaw, A. R. (1989) J. Biol. Chem. 264, 12289–12293
27. Gao, X., Lu, J., and Brugge, J. S. (1997) J. Biol. Chem. 272, 17709–17717
28. Yang, Y., and Frazier, W. A. (1998) Mol. Biol. Cell 9, 309–319
29. Blystone, S. D., Lindberg, F. P., LaFlamme, S. E., and Brown, E. J. (1995) J. Cell Biol. 130, 745–754
30. Cooper, D., Lindberg, F. P., Gamble, J. R., Brown, E. J., and Vadas, M. A. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 3978–3982
31. Wang, X.-Q., and Frazier, W. A. (1998) J. Exp. Med. 188, 21–24
32. Blystone, S. D., Lindberg, F. P., Gillard, M., Prenant, M., and Bouchez, C. (1994) Eur. J. Immunol. 24, 3005–3013
33. Berditchevski, F., Bazzoni, G., and Hemler, M. E. (1995) J. Biol. Chem. 270, 17769–17776
34. Berditchevski, F., Zutter, M. M., and Hemler, M. E. (1996) Mol. Biol. Cell 7, 193–207
35. Zayas-Mo, M., Alfranca, A., Cabanas, C., Marzuela, M., Tejedor, R., Ursa, M. A., Ashman, L. K., de Landazuri, M. O., and Sanchez-Madrid, F. (1998) J. Cell Biol. 141, 791–804
36. Rainger, G. E., Buckley, C., Simmons, D. L., and Nash, G. B. (1997) J. Cell Biol. 131, 1629–1635
37. Dash, S., and Ginsberg, M. H. (1998) Curr. Opin. Cell Biol. 10, 542–550
38. Pearlman, P., Hundi, E., Lawler, J., and Seed, B. (1999) Cell 95, 95–101
39. Tandon, N. N., Lipsky, R. H., Burgess, W. H., and Jamieson, G. A. (1999) J. Biol. Chem. 274, 7570–7575
40. Asch, A. S., Barnwell, J. W., Shattil, S. J., and Brugge, J. S. (1999) J. Biol. Chem. 274, 7576–7583
41. Wang, J. R., Asch, A. S., Silverstein, R. L., and Nachman, R. L. (1999) Blood 84, 1681–1688
42. Savill, J., Nancy, H., Ben, Y., and Haletta, C. (1992) J. Clin. Invest. 90, 1513–1522
43. Raijmakers, S. J., Brouwers, A. M., and van der Heijden, G. J. (1999) J. Biol. Chem. 274, 27300–27309
44. Savill, J., Nancy, H., Nancy, R., and Haletta, C. (1992) J. Clin. Invest. 90, 1513–1522
45. Blystone, S. D., Lindberg, F. P., Brown, E. J. (1994) J. Cell Biol. 127, 1129–1137
46. Savill, J., Nancy, H., Ben, Y., and Haletta, C. (1992) J. Clin. Invest. 90, 1513–1522
47. Ackerman, S. J., and Boulton, A. J. (1997) J. Pathol. 181, 169–182
48. Finnemann, S. C., Bonilha, V. L., Marmorstein, A. D., and Rodriguez-Boulan, E. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 12932–12937
49. Asch, A. S., Barnwell, J. W., Shattil, S. J., and Brugge, J. S. (1999) J. Biol. Chem. 274, 7576–7583
50. Wang, J. R., Asch, A. S., Silverstein, R. L., and Nachman, R. L. (1999) Blood 84, 1681–1688
51. Savill, J., Nancy, H., Ben, Y., and Haletta, C. (1992) J. Clin. Invest. 90, 1513–1522
52. Ackerman, S. J., and Boulton, A. J. (1997) J. Pathol. 181, 169–182
53. Savill, J., Nancy, H., Ben, Y., and Haletta, C. (1992) J. Clin. Invest. 90, 1513–1522
β1 Integrins Associate with CD36

99. Stomski, F. C., Gani, J. S., Bates, R. C., and Burns, G. F. (1992) Exp. Cell Res. 198, 85–92
100. Derakh, D. J., Thorne, R. F., Fecondo, J. V., and Burns, G. F. (1997) J. Biol. Chem. 272, 1323–1330
101. Ren, Y., Silverstein, R. L., Allen, J., and Savill, J. (1995) J. Exp. Med. 181, 1857–1862
102. Franc, N. C., Heitzler, P., Ezekowitz, R. A., and White, K. (1999) Science 284, 1991–1994
103. Savill, J., Dransfield, I., Hogg, N., and Haslett, C. (1996) Nature 343, 170–173
104. Stern, M., Savill, J., and Haslett, C. (1996) Am. J. Pathol. 149, 911–921
105. Fadok, V. A, Warner, M. L, Bratton, D. L., and Henson, P. M. (1998) J. Immunol. 161, 6250–6257
106. Erwig, L. P., Gordon, S., Walsh, G. M., and Rees, A. J. (1999) Blood 93, 1406–1412
107. Coopman, P. J., Thomas, D. M., Gehlsen, K. R., and Mueller, S. C. (1996) Mol. Biol. Cell 7, 1789–1804