CD46 is a ubiquitous human cell surface receptor for the complement components C3b and C4b and for various pathogens, including the measles virus and human herpes virus 6. Ligand binding to CD46 affects (i) protection of autologous cells from complement attack by breakdown of complement components, (ii) intracellular signals that affect the regulation of immune cell function, (iii) antigen presentation, and (iv) down-regulation of cell surface CD46. Recent evidence indicates that CD46 signaling can link innate and acquired immune function. The molecular mechanisms for these processes and the importance of intracellular trafficking of the receptor have not yet been elucidated. We demonstrate here that, in nonlymphoid cells, CD46 is constitutively internalized via clathrin-coated pits, traffics to multivesicular bodies, and is recycled to the cell surface. However, cross-linking of CD46 at the cell surface, by either multivalent antibody or by measles virus, induces pseudopodia that engulf the ligand in a process similar to macropinocytosis, and leads to the degradation of cell surface CD46. Thus, we have elucidated two pathways for CD46 internalization, which are regulated by the valence of cross-linking of CD46 and which utilize either clathrin-coated pits or pseudopodial extension. This has important implications for CD46 signaling, antigen presentation, CD46 down-regulation, and engulfment of pathogens.

CD46 is a human cell surface receptor that regulates innate and acquired immune function. CD46 is expressed on all cells except red blood cells and is a receptor for the complement components C3b and C4b and for pathogens, including measles virus, Neisseria gonorrhoeae and meningitides, Group A streptococcus, and human herpes virus 6 (1–6). Signaling through CD46 affects T cell functions, including increased proliferation of CD3-stimulated cells, suggesting that CD46 might act as a T cell costimulatory molecule (7, 8); immunosuppression in mice of CD3-stimulated cells, suggesting that CD46 might act as a T cell costimulatory molecule (7, 8); and induction of T-regulatory (Tr1) cells (11). Signaling through CD46 can also regulate cytokine production in macrophages (12–15), and binding to CD46 can influence how foreign antigen is presented to the immune system by MHC I Class I or II (16–21). CD46 also plays a critical role in the protection of cells from complement-mediated destruction by acting as a cofactor for the factor-I mediated breakdown of C3b and C4b (4).

An interaction of CD46 with measles hemagglutinin can trigger a rapid down-regulation of CD46 from the cell surface (32). This down-regulation is associated with an increased susceptibility to complement-mediated attack and reduced infectivity of the cell to measles and most likely affects antigen presentation and signal transduction through CD46 (20, 22–29). It is implicitly understood that the internalization of CD46 from the cell surface is important for many of the functions described above, but the pathway by which CD46 is internalized has not yet been elucidated. Indeed, some studies have concluded that CD46 is not internalized (30, 31), although another study has demonstrated internalization of CD46 by immunofluorescence microscopy (32). In support of the notion that CD46 internalization is of biological importance is an observation that the YXXL motif at the juxtamembrane region of the cytoplasm (a motif implicated in internalization of many other proteins) is important for CD46 down-regulation in response to measles infection (23).

Experimental Procedures

Reagents—Cell lines used were CHO-K1 cells (ATCC clone CCL-61), HeLa, M2, L929, Jurkat E6–1, A7-CEM, HPB-TALL, MLA-144, LOVO, COLO-205, U937, U2OS, THP-1, DU145, PC-3-40, K562, Daudi, CCRF CEM, COS7 cells expressing GalU3-3-galactosyl transferase (33), and L229 cells transfected with measles hemagglutinin (L-H cells (26)). Human peripheral T cells were isolated by centrifugation with Ficoll-Paque, adhered to plastic (2 h at 37 °C), and cultured in 800 units/ml human recombinant granulocyte-macrophage colony-stimulating factor (Scherig Plough Ltd., Australia) and 500 units/ml human recombinant IL-4 (PeproTech) in AIM-V (Invitrogen) for 6 days. CHO-K1 cells were transfected with either Superfect (Qiagen) or LipofectAMINE Plus (Invitrogen), selected in Geneticin, and cloned by limiting dilution. COS7 cells were transfected with Superfect Plus (Qiagen).

The abbreviations used are: MHC, major histocompatibility complex; FITC, fluorescein isothiocyanate; PE, phycoerythrin; EGFP, enhanced green fluorescent protein; PBS, phosphate-buffered saline; CHO, Chinese hamster ovary.
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CD46 is and analyzed after 48 h. All cell lines were cultured at 37 °C in 5% CO2.

Monoclonal antibodies to CD46 were E4.3 (affinity-purified IgG2a (34)), M177 (IgG1 (35)), GB24 (IgG1 (36)), and MC120.6 (IgG1 (6)). The 1840 rabbit antibody to CD46 was raised against recombinant extra- cellular domain of CD46, and both protein A-purified and whole serum were used. Other antibodies were to CD71 (BD Pharmingen, San Diego, CA), CD55 (1H4 (37)), CD7 (IgG2a, 1715 (38)), tubulin (IgG1, Sigma), and normal rabbit serum. Secondary antibodies were fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-coupled sheep anti-mouse and sheep anti-rabbit F(ab\(^{-}\))\(_{2}\) antibodies (Sigma) and Alexa594 F(ab\(^{-}\))\(_{2}\) antibodies (Molecular Probes, The Netherlands). The lectin Griffonia simplicifolia B4 (IB-4; Sigma) was biotinylated using standard methods and was detected with Streptavidin-Alexa594 (Molecular Probes, The Netherlands).

CD46 (based on either the pm5.1 clone containing B and C STP exons and the Cyt1 cytoplasmic domain or the pm5.3 clone containing the B STP exon and the Cyt2 cytoplasmic domain (39, 40)) was subcloned into pCDNA3 (Invitrogen), and normal rabbit serum. Secondary antibodies were fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-coupled sheep anti-mouse and sheep anti-rabbit F(ab\(^{-}\))\(_{2}\) antibodies (Sigma) and Alexa594 F(ab\(^{-}\))\(_{2}\) antibodies (Molecular Probes, The Netherlands).

Flow Cytometric Analysis of CD46 Internalization and Down-regulation—All incubations were performed in Dulbecco’s modified Eagle medium containing 10% fetal calf serum and 5.6 mm glutamine. To label surface CD46, cells were incubated either with Alexa-488-E4.3 (10 μg/ml) or with E4.3 (3.9 μg/ml) for 30 min on ice, washed, and incubated with either FITC- or PE-conjugated F(ab\(^{-}\))\(_{2}\) antibody to mouse immunoglobulin (1:10 dilution), and washed again. To allow internalization, cells were incubated in 5% CO2 at 37 °C (or 4 °C as a control). Internalization was stopped by the addition of prechilled medium containing 1.0% sodium azide. To determine the amount of internalized CD46, surface label was removed from the cell surface by incubation with phosphate-buffered saline (PBS) at pH 2.0 for 10 min on ice (Sorvall Strip), and cells were washed three times and resuspended in PBS containing 2% (w/v) glucose, 2% (v/v) formaldehyde and 0.02% sodium azide for analysis by flow cytometry (BD Biosciences). In some experiments, the F(ab\(^{-}\))\(_{2}\) fragment of E4.3 was used without a secondary antibody to ensure monovalent binding. In these instances, intracellular antibody was detected after internalization, acid stripping, fixation, permeabilization, and incubation with a fluoresceinated secondary antibody to mouse immunoglobulin.

To detect the down-regulation of CD46, samples were incubated with 10 μg/ml freshly prepared cycloheximide for 1 h and then incubated with L-H cells at a ratio of 1:1 or antibody as shown. Surface antibody was blocked by a 10 min incubation in PBS at 1:100 dilution, and washed (Molecular Probes). Analysis of CD46 internalization after cross-linking at the cell surface was as described above, except that the fluoresceinated antibody to mouse immunoglobulin was used before internalization at 37 °C. To monitor CD46 internalization after L-H treatment, cells were labeled with the GB24 antibody to CD46 (which does not block F(ab\(^{-}\))\(_{2}\) binding to CD46). Anti-GB24 antibody localization was determined by either direct microscopy (if CD46-EGFP was used) or by fixation, permeabilization, and labeling with Alexa594-coupled antibody to mouse immunoglobulin. The Golgi was detected as previously described, by staining the Golgi3,1Gal epitope after permeabilization with biotinylated 14B4 followed by streptavidin-PE (45). Immunofluorescence was analyzed using an Axiovert Bio-Rad confocal scanning microscope (model MRC1000). The 488-nm line from a krypton/argon laser was used to excite the FITC-tagged secondary antibody, and emission was detected through the 505-nm filter. The 568-nm line from a krypton/argon laser was used to excite the Alexa594-tagged secondary antibody, and emission was detected through the 605-nm filter. Optical sections were recorded at 0.5–1 μm vertical steps, with each image averaged for four scans. All images were processed using the Comos program (Bio-Rad) and Cas 4.02 (confocal assistant software; Todd Clark Brelje). Images are representative of at least two clones of each construct transfected, and staining with subclass control antibodies indicated that the staining was specific.

Transmission Electron Microscopy—The internalization of CD46 was examined with immunoelectron microscopy using three different cell lines: CHO-K1 stably transfected with CD46, COS7 cells transiently transfected with CD46, and HeLa cells expressing endogenous CD46, all of which gave similar results. Cells were labeled with antibodies against CD46 either prior to or after chemical fixation. Antibodies to transfected cells were performed between 1840 or E4.3 (2 μg/ml). As controls, cells were also labeled with antibody to CD565 (55 μg/ml), CD71 (IgG2a; 5 μg/ml), and CD7 (IgG2a; 1:1000 dilution) either before or after chemical fixation. For prefixation labeling experiments, cells were exposed to the antibody at 4 °C and then incubated at 37 °C for 30 or 60 min. Cells were fixed in 2% paraformaldehyde and 0.05% glutaraldehyde in 0.08 μg/ml Sorenson’s phosphate buffer, pH 7.4, for 30 min at room temperature, rinsed in 0.8 μg/ml Sorenson’s phosphate buffer with 5% sucrose, free aldehydes were quenched, and cells were permeabilized with 0.1% freshly prepared sodium borohydride in 0.08 μg/ml Sorenson’s phosphate buffer. Cells were incubated in blocking buffer (5% bovine serum albumin, 0.1% normal goat serum in PBS) for 1 h. Cells not exposed to a primary antibody prior to fixation were incubated with the antibody dilutions mentioned above and incubated overnight at 4 °C. Cells were incubated overnight at 4 °C with the (Fab\(^{-}\))\(_{2}\) fragments of goat IgG against mouse immunoglobulin conjugated to UltraSmall Gold (Aurion) diluted 1:500 in incubation buffer (0.8% bovine serum albumin, 0.1% cold water fish skin gelatin in PBS, pH 7.2), and washed thoroughly in PBS buffer prior to exposure to an UltraSmall Gold (Aurion) diluted 1:500 in PBS, coated with a thin layer of uranyl acetate and lead citrate and examined in a Hitachi H600 Transmission Electron Microscope.

SDS-PAGE—HeLa cells were incubated in 1840 (2 μg/ml) or normal rabbit serum for 30 min on ice at 37 °C. Cells were lysed at 107–108 cells/ml in 140 mM NaCl, 0.5% Nonidet P-40, 10 mM Tris, pH 8.0, 5 mM EDTA with Complete protease inhibitors (Roche Applied Science). Lysates were electrophoresed on a 10% SDS-PAGE gel in nonreducing conditions, transferred and probed with E4.3 (3.9 μg/ml), stripped, and reprobed with antibody to tubulin (1:5,000).

Measles Virus Binding and Internalization—Cells were harvested from nonconfluent monolayers, washed in Dulbecco’s modified Eagle medium containing 10% fetal calf serum, and then incubated with purified MV Haltéf (at doses previously found to saturate CD46 binding (44)) for 30 min at 37 °C in 5% CO2. Cells were then centrifuged at 1,300 rpm for 3 min, and the cell pellets were resuspended in 1 ml of fresh 2.5% glutaraldehyde (in 0.08 μg/ml Sorenson’s phosphate buffer pH 7.4) and incubated at room temperature for 30 min. After centrifuging at 1,300 rpm for 3 min, cells were resuspended in 1 ml of 0.8 μg/ml Sorenson’s phosphate buffer with 5% sucrose, pH 7.4, prior to analysis by transmission electron microscopy.

Yeast Two-hybrid Assay—Screening and validation of the interaction between CD46 and AP-2 was performed as described in Ref. 45. Sequences encoded by the GAL4-BD fusion constructs were PYR1LQRK-RKKGTKLYTIDTDEHREVKFTSL and PYRLQRKKKGGKDGAAYEATYQTKSTTPAEQGG for Cyt1 and Cyt2, respectively.

RESULTS

CD46 Internalizes without Cross-linking—To measure the internalization of CD46, cells were labeled with saturating amounts of FITC-labeled E4.3 antibody to CD46 at 4 °C. Following incubation at either 4 or 37 °C for 30 min, the internalized antibody was quantified by comparing fluorescence levels with or without acid removal of surface-bound antibody (“acid stripping”). Antibodies to CD46 were internalized from the surface of HeLa cells (Fig. 1A, i) but not from the T cell line MLA-144 (Fig. 1A, ii), as indicated by protection from acid stripping after incubation at 37 °C (solid line) compared with...
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CD46 internalizes on nonlymphoid human cell types

A panel of cells and cell lines was assayed for CD46 internalization. Cell surface CD46 was labeled with E4.3 and a FITC-conjugated secondary antibody at 4 °C. Cells were then incubated at 37 °C for 30 min to allow internalization. Following acid stripping of remaining surface antibody, an increase in fluorescence from 4 to 37 °C by flow cytometry indicated the internalization of CD46 and was scored as “+”; no increase in fluorescence indicated that CD46 had not internalized and was scored as “−.”

| Cell type          | Cell line | Constitutive CD46 internalization (pathway 1) |
|--------------------|-----------|---------------------------------------------|
| Cervical adenocarcinoma | HeLa-M    | +                                           |
| Colon adenocarcinoma     | LOVO      | +                                           |
| Colon adenocarcinoma     | COLO-205  | +                                           |
| Prostate adenocarcinoma  | DU145     | +                                           |
| Prostate adenocarcinoma  | PC-340    | +                                           |
| Melanoma               | M2        | +                                           |
| Monocyte leukemia       | THP-1     | +                                           |
| Myeloid leukemia        | U937      | +                                           |
| Promyeloid leukemia      | HL-60     | +                                           |
| Myeloid leukemia         | K562      | +                                           |
| Astrocytoma             | U251MG    | +                                           |
| Primary dendritic cells  |           | +                                           |
| Peripheral blood lymphocytes |          | −                                           |
| B cell lymphoma         | Daudi     | −                                           |
| T cell leukemia         | Jurkat-E61| −                                           |
| T cell leukemia         | CCRF-CEM  | −                                           |
| T cell leukemia         | HPB-TALL  | −                                           |

4 °C (dotted line). Similar fluorescence was seen between non-acid-treated cells at 4 and 37 °C, indicating that antibody was not shed from the cell surface (Fig. 1A, iii and iv). CD46 was internalized from all nonlymphocyte cell types tested, but little or no internalization after 30 min was detected from the surface of human peripheral lymphocytes or lymphocyte cell lines (Table I). Internalization was evident by 15 min and peaked within 30–60 min, and both Cyt1 and Cyt2 isoforms of CD46 gave identical patterns of internalization (data not shown). The antibody internalization was specifically dependent upon CD46, since control antibodies, antibodies to CD46 incubated with untransfected CHO-K1 cells, and an antibody to the multidrug resistance protein Pgp bound to A7-CEM cells were not internalized at this time point (data not shown). These data indicate that noncross-linked CD46 internalizes from the surface of nonlymphocyte cells but not from the surface of lymphocytes.

CD46 internalization was also observed with Fab′) fragments of E4.3 and with monoclonal antibodies directed to different epitopes of CD46 (M177, 20.6; data not shown), suggesting that CD46 internalization might be a constitutive process. We reasoned that, if the internalization of CD46 was a steady-state process not affected by the E4.3 antibody, treatment with E4.3 should not cause a down-regulation of CD46 at the cell surface. We therefore investigated whether incubation with the E4.3 antibody altered the surface expression of CD46 on HeLa cells (Fig. 1B). As a positive control for CD46 down-regulation, cells were incubated for 30 min at 37 °C with L929 cells expressing measles virus hemagglutinin (L-H cells). As previously described (26), incubation with L-H caused an extensive reduction in surface CD46 on the HeLa cells (Fig. 1B, ii). E4.3 antibody altered the surface expression of CD46 on HeLa cells (Fig. 1B, i, solid line) compared with incubation with untransfected L929 cells (Fig. 1B, i, dotted line). In contrast, the surface expression of CD46 on HeLa cells (Fig. 1B, ii, dotted line) was not reduced by E4.3 incubation at 37 °C for 30 min (Fig. 1B, ii, solid line), despite this antibody being internalized (data not shown). Extending the incubation time to 4 h still had no effect on surface CD46 expression (data not shown), and the maintenance of CD46 surface expression was not due to new protein synthesis, since the cells were incubated with cycloheximide. These data
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Internalized CD46 Colocalizes with the Golgi Apparatus—E4.3 antibody that had been internalized localized to a single region within the CHO cells transfected with CD46 (Fig. 1C, i). This staining was strikingly similar to the steady state localization of CD46 in these cells (Fig. 1C, ii), suggesting that internalized CD46 might traffic to the region of steady state CD46 localization. To test this hypothesis, we generated a CD46-EGFP fusion protein in which the Ser/Thr/Pro-rich extracellular stalk of CD46 was replaced with EGFP. CD46-EGFP had the same intracellular distribution and trafficking characteristics as wild type CD46, as determined by flow cytometric analysis (data not shown), steady state CD46-EGFP fluorescence (Fig. 1C, iii), and localization of internalized E4.3 antibody (Fig. 1C, iv). Importantly, the internalized antibody (Fig. 1C, iv, and red in Fig. 1C, v) colocalized with the CD46-EGFP fluorescence (Fig. 1C, iii, and green in Fig. 1C, v), indicating that internalized CD46 trafficked to the region of constitutive expression of CD46. These data indicate that intracellular CD46 is composed, at least in part, of CD46 that has been internalized from the cell surface.

Colocalization studies were performed to further define the location of intracellular CD46. First, cells were fixed, permeabilized, and costained with antibodies to CD46 and propidium iodide, which showed that intracellular CD46 was not in the nucleus (data not shown). Second, to detect the Golgi, we used the IB4 lectin, which binds to the Galα1,3Gal moiety of glycoconjugates. This linkage is produced by the α1-3-galactosyltransferase, which resides in the Golgi (43). As expected for a protein that is processed through the secretory pathway, the intracellular CD46 in transfected COS7 cells (Fig. 1D, i, and green in Fig. 1D, iii) was colocalized in part with the Golgi apparatus (determined by IB4 labeling) (Fig. 1D, ii, and red in Fig. 1D, iii). Thus, at least some of the CD46 is either in transit through the Golgi during processing or is internalized into a Golgi compartment. As previously seen in CHO cells, part of the intracellular pool of CD46 (Fig. 1D, iv, and green in Fig. 1D, vi) was in the same region as internalized CD46 (Fig. 1D, v, and red in Fig. 1D, vi). These data show that internalized CD46 traffics to a compartment in the vicinity of the Golgi but do not provide the resolution to determine whether this compartment is the Golgi per se. Intracellular CD46 staining was only partially abrogated by cycloheximide treatment and partially dispersed by brefeldin A treatment, whereas IB4 staining was dispersed (data not shown), suggesting that some but not all of the CD46 was newly processed protein in Golgi vesicles. These data indicate that after internalization, CD46 traffics to a storage site at or near the Golgi apparatus.

Internalized CD46 Traffics to Multivesicular Bodies—To more precisely define the localization of internalized CD46, we performed immunoelectron microscopy on HeLa cells. Antibody to CD46 labeled clusters of multivesicular bodies (Fig. 2A, i and ii). These multivesicular bodies were often perinuclear and in the vicinity of Golgi stacks (Fig. 2A, iii). This staining is in

antibodies to CD46 (E4.3; i and ii), CD71 (iii), and CD55 (iv) at 37 °C for 60 min, fixed, permeabilized, and labeled with a gold-coupled antibody to mouse immunoglobulin for immunoelectron microscopy. C, untreated HeLa cells were fixed, permeabilized, and immunolabeled with the E4.3 antibody to CD46 followed by gold-coupled antibody to mouse immunoglobulin. Scale bars represent 250 nm (A, i and ii), 500 nm (A, iii), or 100 nm (B and C); asterisks indicate Golgi apparatus; and N indicates the nucleus.

Fig. 2. CD46 is constitutively internalized via clathrin-coated pits and traffics to multivesicular bodies. A, HeLa (i and ii) and CHO-K1 cells transfected with CD46 (B2 isoform) (iii) were incubated with the E4.3 antibody to CD46 at 37 °C for 60 min, fixed, permeabilized, and labeled with a gold-coupled antibody to mouse immunoglobulin for immunoelectron microscopy. B, HeLa cells were incubated with indicate that the loss of CD46 from the cell surface through internalization is compensated by a gain in CD46 from an intracellular storage compartment and are compatible with the notion that CD46 is constitutively cycled between the cell surface and an intracellular compartment.
agreement with our coimmunofluorescent studies and is reminiscent of the recycling vesicles observed with the transferrin receptor and epidermal growth factor receptor, which are often near the Golgi apparatus (46, 47).

**CD46 Internalizes via Clathrin-coated Pits and Interacts with the Clathrin Adaptor Protein AP-2**—The transferrin receptor traffics to multivesicular bodies via clathrin-coated pits. To determine whether CD46 internalization uses a similar mechanism, HeLa cells were incubated with the E4.3 antibody to CD46 for 60 min at 37 °C, fixed, permeabilized, and labeled with a gold-coupled antibody to mouse immunoglobulin. Antibody to CD46 was observed both in clathrin-coated pits budding from the cell surface (Fig. 2B, i) and in internalized clathrin-coated pits (Fig. 2B, ii). Controls for labeling included antibodies to CD71 (known to internalize through clathrin-coated pits) (Fig. 2B, iii) and antibodies to CD59 (a glycosylphosphatidylinositol-linked membrane protein that internalizes via caveolae) (Fig. 2B, iv), which were present on clathrin-coated pits and smaller, less electron-dense vesicles, respectively. Clathrin-coated pits were also seen when either CHO-K1 or COS7 cells transfected with CD46 were incubated with the E4.3 antibody (data not shown). To determine the trafficking of CD46 itself, we fixed and permeabilized untreated HeLa cells and immunolabeled with the E4.3 antibody to CD46. CD46 was observed in the clathrin-coated pits of these cells (Fig. 2C, i) and in internalized, clathrin-coated vesicles (Fig. 2C, ii), but the negative control antibody, 1715, did not label clathrin-coated pits (data not shown). These data indicate that the localization of internalized antibodies to CD46 corresponds to the localization of CD46 itself. Furthermore, the internalization of CD46 via clathrin-coated pits is a constitutive process, occurring without the addition of exogenous ligand.

CD46 is alternatively spliced to encode two different cytoplasmic domains (Cyt1 and Cyt2), which are coexpressed in all cell types (40). Previously, a yeast two-hybrid screen showed that the cytoplasmic domain 1 of CD46 interacted with the cell polarity protein, Discs large (45). Here, we show that both cytoplasmic domains of CD46 associate with the AP-2 adaptor protein of clathrin-coated pits. The interacting fusion construct encoded protein containing, in frame, the FGYPQ domain of the μ subunit, known to bind to Y- and L-based sorting motifs (48). Cells grown, indicating an interaction, was observed when AP-2 was cotransformed with CD46-Cyt1 (Fig. 3A, i) or CD46-Cyt2 (Fig. 3A, ii), but not when AP-2 was cotransformed with the C-terminal region of cytoplasmic tail 2, missing the YXXL domain (Fig. 3A, iii). The interaction between AP-2 and CD46 detected in the two-hybrid system was specific, because cells transformed with AP-2 and pAS2.1 (Fig. 3A, iv) and pLAM (human laminin C) (Fig. 3A, v) did not grow. Growth of all transformants on medium deficient in Trp and Leu indicated that both plasmids were expressed (Fig. 3B, i–v).

These data indicated that CD46 and AP-2 were associated via the 12-amino acid sequence common to both CD46 cytoplasmic tails. These 12 amino acids contain the sequence YRYL, which is of interest for two reasons. First, this sequence corresponds to the consensus YXXL motif known to be important for association of many other proteins with AP-2, for internalization via clathrin-coated pits, and for sorting to the multivesicular body (49). Second, this sequence has previously been shown to be required for the down-regulation of CD46 in cells constitutively infected with the measles virus, and it has been postulated that this was due to effects on internalization of CD46 (23). These data combined suggest that the YRYL sequence of CD46 mediates internalization in clathrin-coated pits via an interaction with AP-2 in a similar manner to the transferrin receptor. In support of this hypothesis is the observation that AP-2 was cotransformed with CD46 (48). Cell growth, indicating an interaction, was observed when the C-terminal region of cytoplasmic tail 2, missing the YRYL sequence, did not grow (Fig. 3B, v). In support of this hypothesis is the observation that the negative control antibody, 1715, did not label clathrin-coated pits (data not shown). These data indicate that the localization of internalized antibodies to CD46 corresponds to the localization of CD46 itself. Furthermore, the internalization of CD46 via clathrin-coated pits is a constitutive process, occurring without the addition of exogenous ligand.

Yeast colonies were subjected to growth tests on selective medium deficient in Leu, Trp, and His to determine an interaction between the two transformed fusion proteins (A) or deficient in Leu and Trp to demonstrate effective transformation of the two fusion proteins (B). COS7 cells were transfected with CD46, incubated with E4.3 for 1 h at 37 °C (i and red in iii) and FITC-coupled transferrin for 1 h at 37 °C (ii and green in iii), fixed, permeabilized, and immunolabeled with Anti-exa594-coupled antibody to mouse immunoglobulin. White indicates regions in which the red and green stains were colocalized according to the Colocalization plug-in of the Image J analysis software (National Institutes of Health), with the ratio set at 50% and the threshold levels at 80.

CD46 (Fig. 3C, i, and red in Fig. 3C, iii) and the transferrin receptor (Fig. 3C, ii, and green in Fig. 3C, iii) are both internalized to a similar perinuclear region. Internalized CD46 in COS7 cells were not clustered as tightly as in CHO cells and were visible throughout the cell. However, the vesicles clearly concentrated toward the middle of the cell, and the Image J Colocalization plug-in (National Institutes of Health) (white in Fig. 3C, iii) indicated that some, but not all, vesicles in this region contained both CD46 and transferrin receptor. Thus, CD46 associates with the clathrin-coated pit adaptor molecule AP-2 and is constitutively internalized via clathrin-coated pits, traffics to a multivesicular storage compartment near the Golgi apparatus, and is recycled to the cell surface. This process of internalization was termed “Pathway 1.”

**Cross-linking of CD46 Alters the Mechanism of Internalization**—During these investigations, we noted that Pathway 1 of CD46 internalization was only observed when the cells were
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Fig. 4. Cross-linking of cell surface CD46 alters the trafficking of internalized CD46. A, CHO-K1 cells transfected with wild-type CD46 (B2 isoform) were incubated at 37°C with E4.3 for 60 min (i) or E4.3 and a sheep (Fab′)2 secondary antibody to mouse immunoglobulin for 15 min (ii) or 60 min (iii). Cells were fixed, permeabilized, stained with Alexa594-labeled antibody to mouse immunoglobulin, and viewed under confocal microscopy. B, CHO-K1 cells stably expressing CD46-EGFP (green, Cyt2 isoform) were incubated at 37°C with E4.3 (i) or E4.3 and sheep antibody to mouse immunoglobulin (ii) for 1 h, fixed, permeabilized, stained with Alexa594-labeled antibody to mouse immunoglobulin (red), and analyzed by confocal microscopy. Levels of red and green fluorescence across the white lines in i and ii are shown in iii and iv, respectively. C, CHO-K1 cells transfected with CD46 were labeled at 4°C with the GB24 monoclonal antibody to CD46 (which does not interfere with the binding of CD46 to measles hemagglutinin) and then incubated with L-H cells for 8 h at 37°C. Cells were fixed, permeabilized, stained with Alexa594-labeled antibody to mouse immunoglobulin to detect the GB24 antibody, and analyzed by confocal microscopy.

incubated with monoclonal antibodies that had not been cross-linked by secondary antibodies. A strikingly different pattern of internalization was seen when cells were incubated with monoclonal antibodies that had been cross-linked with secondary antibodies or were incubated with a polyclonal antibody to CD46 (Pathway 2). In contrast to the pattern seen with mono- or bivalent antibody (Pathway 1; Figs. 1C and 4A, i), incubation of cells with cross-linked E4.3 resulted in the trafficking of E4.3 to discrete intracellular vesicles. These vesicles were most evident near the plasma membrane after 15 min (Fig. 4A, ii) and converged toward the center of the cell by 1 h but remained clearly discrete (Fig. 4A, iii).

CHO-K1 cells expressing CD46-EGFP were used to correlate the internalized CD46 with the steady state CD46. Again, uncross-linked CD46 was internalized to the same intracellular region as the constitutive CD46 (Fig. 4B, i), and a histogram representing the fluorescence intensities across an individual cell shows superimposed red and green peaks (Fig. 4B, iii). In contrast, EGFP-CD46 that had been internalized after cross-linking with E4.3 and antibody to mouse immunoglobulin was observed in vesicles that aggregated toward, but remained distinct from, the constitutive intracellular CD46 (Fig. 4B, ii). In this case, the red histogram contained multiple peaks, which did not superimpose with the green histogram (Fig. 4B, iv).

Importantly, treatment with the 1840 polyclonal antibody to CD46, which would also be expected to cause multivalent cross-linking, produced a staining pattern identical to that induced by cross-linked E4.3 (data not shown). Because the known physiological ligands to CD46 are multivalent (e.g., complement-fixed particles or virus containing multiple hemagglutinin molecules), we tested whether cross-linking CD46 with antibodies might have the same effect as cross-linking CD46 with physiological ligands. We therefore investigated the pattern of internalization induced by L-H cells (L929 cells expressing the measles virus hemagglutinin protein). These cells have been used extensively to analyze the effect of measles virus on CD46 function and mimic the effect of the measles virus in causing CD46 down-regulation (26). Indeed, incubation of CHO-K1 cells expressing CD46 with L-H cells induced a pattern of CD46 internalization similar to that seen with either cross-linked E4.3 or the polyclonal antibody, 1840 (Fig. 4C).

Furthermore, expression of MV-H protein in HeLa cells resulted in the same intracellular staining pattern of CD46 (32). These data indicate that CD46 can internalize by two pathways and suggest that the pathway is determined by the extent of cross-linking of CD46.

CD46 Internalization via Pathway 2 Leads to CD46 Down-regulation—We compared the surface expression of CD46 on Jurkat cells (Fig. 5A) that had been incubated for 4 h with (i) no stimulus, (ii) L-H cells, (iii) the E4.3 monoclonal antibody to CD46 (without cross-linking), or (iv) the 1840 polyclonal antibody to CD46. As expected, L-H caused the down-regulation of CD46 surface expression, and treatment with E4.3 alone had no effect on CD46 surface expression. Treatment with 1840 (iv) resulted in a loss of surface expression of CD46 similar to that seen after treatment with L-H cells. Thus, cross-linking of CD46 by either multivalent antibody or by treatment with multivalent measles hemagglutinin, which cause internalization through Pathway 2, results in down-regulation of CD46. In contrast, treatment with noncross-linking antibody, which involves internalization through Pathway 1, does not result in the down-regulation of CD46.

To confirm that the choice of pathway was determined by the valence of cross-linking, we compared treatments in which the same antibody (E4.3) to CD46 was bound at the same concentration, but the valence of cross-linking was changed using different concentrations of secondary antibody to mouse immunoglobulin. Indeed, although low concentrations of secondary antibody did not induce CD46 down-regulation (Fig. 5B, i), increasing concentrations of secondary antibody resulted in a dose-dependent increase in the down-regulation of CD46 (Fig. 5B, ii and iii). These differences were not due to a lack of
binding or internalization, since significant binding of the antibody was detected in all cases (data not shown), and this antibody was internalized and protected from acid stripping in all cases (Fig. 5B, iv–vi). Similarly, the 1840 antibody had differential effects on down-regulation, depending upon the concentration of the antibody, with low concentrations unable to induce down-regulation although the antibody was internalized (data not shown). These data indicate that concentrations of antibody to CD46 that mediate low valence binding do not divert the internalization pathway, so CD46 is internalized via Pathway 1 and is not down-regulated. In contrast, higher concentrations of antibody allow multivalent cross-linking and thus induce Pathway 2 and trigger the down-regulation of CD46.

**Down-regulation of Surface CD46 via Pathway 2 Results in Depletion of CD46 from Multivesicular Bodies**—Our investigations into the trafficking of CD46 through Pathway 1 suggested that the pool of CD46 present in multivesicular bodies is in dynamic equilibrium with the cell surface CD46 and that CD46 from multivesicular bodies is continually recycled to the cell surface. Therefore, the down-regulation of surface CD46 that we and others (24–26, 32, 50) observed after cross-linking must be accompanied by a reduction in the CD46 in multivesicular bodies. To test this possibility, we first investigated the effect of

**Fig. 5. Cross-linking of cell surface CD46 correlates with CD46 down-regulation.** A, Jurkat cells were incubated at 37 °C for 4 h (solid histograms) with nothing (i), L-H cells (ii), E4.3 (iii), or 1840 (iv). Residual surface antibody was removed by treatment with PBS (pH 2.0), and CD46 was stained with 1840 and a PE-labeled F(ab')₂ antibody to rabbit immunoglobulin before cells were analyzed by flow cytometry. Controls (dotted histograms) were treated with the same antibodies at 4 °C (i, iii, and iv) or with untransfected L929 cells at 37 °C (ii). B, CHO-K1 cells transfected with wild-type CD46 were incubated at 4 °C (dotted histograms) or 37 °C for 30 min (solid histograms) with E4.3 and FITC-labeled F(ab')₂ secondary antibody to mouse immunoglobulin at the concentrations indicated (i–vi). Surface-bound antibody was removed with PBS (pH 2.0), and cell surface CD46 was stained with E4.3 and a PE-labeled F(ab')₂ antibody to mouse immunoglobulin to monitor the amount of CD46 remaining at the cell surface. Cell surface (PE staining, i–iii) and internalized antibody (FITC retention, iv–vi) was analyzed by flow cytometry. All cells were pretreated with cycloheximide to prevent new protein synthesis. Note that the increased fluorescence of cells incubated at 4 °C in vi is due to inefficient acid stripping routinely seen at these high levels of labeling and does not reflect internalized protein. C, HeLa cells treated with 1840 or normal rabbit serum for 30 min or 4 h at 37 °C were lysed and quantified for CD46 expression by immunoblotting. Protein loading was determined by immunoblotting with antibody to tubulin. D, CHO-R1 cells transfected with CD46-EGFP cells were incubated with L-H cells at 37 °C for 0 min (i), 15 min (ii), 1 h (iii), and 4 h (iv) and analyzed by confocal microscopy.
cross-linking on the total cellular pool of CD46 using immunoblotting (Fig. 5C). Treatment of HeLa cells with the 1840 antibody almost completely abrogated CD46 expression by 4 h (compare lane 2 with lane 4), suggesting that both the cell surface and the intracellular pool of CD46 had been degraded. We next investigated the effect of surface cross-linking on the intracellular CD46-EGFP. CHO-K1 cells transfected with CD46-EGFP (Fig. 5D, i) were incubated with L-H cells expressing measles hemagglutinin protein for the indicated time points, and CD46-EGFP fluorescence was assessed by confocal microscopy. Within 15 min (Fig. 5D, ii), the expression of cell surface CD46 was reduced, and the expression of intracellular CD46 was slightly increased. However, after 1 h (Fig. 5D, iii) and 4 h (Fig. 5D, iv), CD46 both on the cell surface and in the perinuclear area was substantially decreased. These data indicate that cross-linking of CD46 at the cell surface not only affects cell surface CD46 but also diverts CD46 from storage in multivesicular bodies. A likely explanation for this observation is that CD46 rapidly exchanges between multivesicular bodies and the cell surface, so that CD46 that has constitutively internalized via Pathway 1 will rapidly recycle back to the cell surface, where it can be cross-linked and diverted through Pathway 2 to be degraded.

CD46 Internalization via Pathway 2 Utilizes the Induction of Pseudopodia—We used immunoelectron microscopy to determine the ultrastructural characteristics of Pathway 2. Incubation of CHO-K1 cells expressing CD46 with the 1840 polyclonal antibody did not result in the labeling of clathrin-coated pits. Instead, CD46 was clustered near pseudopodia or membrane ruffles, which fold over to enclose the CD46 in vesicles of ~0.5–1 μm in diameter (Fig. 6, i–iii). CD46 was internalized into these vesicles irrespective of whether it was cross-linked by 1840 (i–iii) or by E4.3 and antibody to mouse immunoglobulin (Fig. 6iv). This pattern of internalization was not observed for E4.3 that had not been cross-linked (Fig. 3 and data not shown). Gold-labeled vesicles were evident near the nucleus, but multivesicular bodies were not labeled, indicating that CD46 internalized via Pathway 2 was transported in vesicles that moved toward the nucleus but did not fuse with multivesicular bodies (Fig. 6v). The morphology of the pseudopodia and the size of the vesicles were most similar to those described for macropinocytosis, a process induced by extracellular stimuli in various cell types (51–53). These data indicate that CD46 internalization via Pathway 2 involves the induction of pseudopodia and not clathrin-coated pits.

We next tested whether Pathway 2 could be induced by measles virus infection. HeLa cells were incubated with the Hallé strain of measles virus for 30 min and fixed to ensure that the virus was captured in the process of entering the cell rather than budding off from the cell. First, incubation with measles virus resulted in an increase in the density of pseudopodia, and this increase was not seen on CHO-K1 cells that did not express CD46, indicating that specific binding to CD46 was required for this effect. Strikingly, concentrations of virus that saturated CD46 binding triggered a dramatic change to the morphology of both transfected CHO-K1 (data not shown) and HeLa cells (Fig. 7, compare i with ii–iv), with large numbers of pseudopodia clustered toward one pole of the cell away from the nucleus (Fig. 7, ii–iv). Thus, cross-linking of CD46 by both 1840 or measles virus can induce the production of pseudopodia.

These observations raise the possibility that measles virus (or other pathogens that interact directly or indirectly with CD46) might internalize via macropinocytosis. The primary mechanism for measles internalization is through viral fusion, but alternative pathways may exist to remove fusion-incompetent virus from the cell surface. Measles virus is difficult to discriminate morphologically, so we could not conclusively identify viral particles. However, increasing numbers of particles that were of a size and shape compatible with measles virus were observed as cells were incubated with increasing amounts of virus (data not shown). These particles were often associated with pseudopodia and looked to be in the process of internalization by macropinocytosis (Fig. 7, i–iii). These data combined indicate that the cross-linking of CD46 at the cell surface by polyclonal antibody or measles virus triggers a process similar to macropinocytosis.

**DISCUSSION**

We describe here the first comprehensive analysis of the mechanisms by which CD46 is internalized and show that internalized CD46 can follow one of two different intracellular pathways depending upon the valence of ligand binding at the cell surface and upon the cell type (Fig. 8). In nonlymphoid cells, CD46 constitutively internalizes from the cell surface via clathrin-coated pits, migrates to multivesicular bodies, and is recycled back to the cell surface (Pathway 1). However, cross-linking of CD46 at the cell surface can divert internalization to a different pathway, which is independent of clathrin-coated pits, leads to protein degradation, and resembles macropinocytosis (Pathway 2). This pathway occurs in nonlymphoid cells, as reported here, and probably in lymphoid cells, as shown by the efficient CD46 down-regulation previously observed in Jurkat cells incubated with L-H.
cells (26) (data not shown). These pathways have a number of implications for the biology of CD46.

CD46 and Antigen Presentation—The constitutive internalization and recycling observed here for CD46 (Pathway 1) is a common feature of proteins whose functions include either nutritional uptake (e.g. the transferrin receptor) or a sampling of the extracellular environment (e.g. monitoring of extracellular lipid levels by the low density lipoprotein receptor or providing extracellular proteins for antigen presentation by IgR (54)). The constitutive internalization of CD46 may be related to its role in the regulation of antigen presentation. The multivesicular bodies to which CD46 constitutively traffics are morphologically similar to the antigen loading compartment for Class I (and to some extent Class II) MHC (termed MIIC (55)). Interestingly, measles virus fusion protein has been localized with Class I MHC in the MIIC of a measles-infected B cell model system (56, 57), and CD46 cocaps with and has been copurified with Class I MHIC (58), suggesting a physical association. Furthermore, the expression of CD46 can affect the processing of MHC-bound ligands, either by increasing the efficiency of presentation or by diverting peptides to presentation by Class II (16–19, 21). However, the route of internalization may have a more complex impact upon presentation, since macropinocytosis can also enhance the presentation of antigen via MHC and can affect the choice of presentation via Class I or Class II (59–61). The elucidation of two pathways for CD46 internalization provides a basis from which to dissect the influence of cross-linking on antigen presentation, by comparing the presentation of monomeric and multimeric ligands and comparing the effects of inhibitors of the two internalization pathways on antigen presentation. These data also suggest the possibility that CD46 plays a more general role in sampling the

![FIG. 7. Incubation with measles virus induces pseudopodia. HeLa (i–iv, vi, and vii) or CHO-K1 transfected with CD46 (BC1) (v) were incubated with no virus (i) or saturating concentrations of measles virus (ii–vii) for 30 min, fixed, and analyzed by electron microscopy. Scale bar, 5.0 µm (i–iv) and 0.5 µm (v–vii).](image)

![FIG. 8. Schematic of the two pathways by which CD46 can internalize. The left hand side depicts the constitutive internalization of CD46 through clathrin-coated pits and the recycling of CD46 through multivesicular bodies. The right hand side depicts the macropinocytosis-like internalization induced by cross-linking of CD46. CD46 internalized in this manner is diverted from clathrin-coated pits and from multivesicular bodies, resulting in substantial down-regulation of CD46.](image)
extracellular environment, perhaps providing complement-bound particles for presentation on MHC.

**Internalization and Signaling**—The nature and extent of signaling through a cell surface receptor is often inextricably linked to the internalization characteristics of the receptor (62). For instance, signaling from many receptors is reduced by the internalization and subsequent degradation of the receptor, but the signaling of the extracellular growth factor receptor is facilitated by internalization into endosomes (63). Signaling through CD46 is induced by receptor cross-linking (via antibody, dimerized complement, and measles) (8, 11, 12) and therefore is likely to correlate with Pathway 2 observed here. The observation that this pathway leads to the down-regulation of CD46 suggests an intrinsic negative feedback mechanism that regulates CD46 signaling. Interestingly, the polarized macropinocytosis observed here and the morphological changes induced in T cells by CD46 signaling (7) are compatible with the observation that many of the proteins implicated in CD46 signaling are also involved in membrane trafficking and cell morphology (Vav, Rac, Cbl, Dlg) (7, 8, 45) and suggest that a major effect of CD46 signaling is to reorganize the cell shape.

The lack of constitutive internalization of CD46 in lymphocytes is reminiscent of CD4, which is constitutively internalized and recycled to the cell surface in CD4-transfected nonlymphocyte cell lines and in monocytes and macrophages but is prevented from internalizing in many lymphocyte cell types by binding of p56lck (64, 65). During T cell activation, phosphorylation of CD4 allows dissociation of p56lck and internalization of the receptor (66). CD46 also associates with Src family kinases, including p56lck (67, 68), and it will be interesting to determine whether a similar mechanism controls the internalization of CD46.

**CD46 as a Regulator of Macropinocytosis**—Our experiments show that the cross-linking of CD46 is not a cause of internalization, as might have previously been thought, but diverts CD46 to a different route of internalization. Pathway 2 involves the extension of membrane ruffles or pseudopodia, which end in small particles (60). Macropinocytosis can be induced in many cell types by soluble mediators such as epidermal growth factor, and internalization of the receptor (66). CD46 also associates with Src family kinases, including p56lck (67, 68), and it will be interesting to determine whether a similar mechanism controls the internalization of CD46.

Down-regulation of CD46 correlates with the induction of Pathway 2 internalization and macropinocytosis. Down-regulation of CD46 can be induced by measles infection, measles hemagglutinin expressed on L cells, complement activation, polyclonal antibodies to CD46, and monoclonal antibodies when aggregated with a secondary antibody but not by monoclonal antibodies alone or “cross-linking” antibodies used at low concentrations to prevent aggregation (see Refs. 24 and 26, and see above). The molecular events involved in the internalization of CD46 have primarily been defined here using antibodies so that the valence of binding can be controlled, and these experiments indicate that down-regulation of CD46 is caused by an aggregation-dependent redirection of CD46 trafficking. Thus, either an intact measles virus (containing many hemagglutinin molecules which are tetrameric (71)) or a complement-opsonized particle would replicate the aggregation triggered by cross-linked antibody and induce Pathway 2. This may facilitate engulfment of these particles. Indeed, we show here that measles virus can induce similar extensions of pseudopodia in CD46-expressing cells, which provides an opportunity for ingestion. Although MV infection of host cells proceeds from the fusion of the virus envelope with the plasma membrane at neutral pH, it can be inferred from these data that MV particles may also enter cells during macropinocytosis.

The pseudopodia triggered by 1840 cross-linking and incubation with measles virus are morphologically similar to those triggered by the binding of N. meningitidis pili to human umbilical vein endothelial cells (72). The ErbB2 receptor is necessary, but not sufficient, for the induction of pseudopodia after Neisseria binding (73). CD46 can bind to the pili of N. meningitidis (3) and facilitate transmigration across the blood-brain barrier (74). Our data suggest that CD46 might facilitate the internalization of Neisseria by macropinocytosis. Thus, it is possible that CD46 can trigger the internalization of small multivalent particles but must cooperate with other receptors to allow the internalization of larger multivalent particles. The induction of pseudopodia may enhance the attachment of Neisseria to cells, thus explaining the loss of adherence when the cytoplasmic domain of CD46 is deleted (75). The effect of cross-linking CD46 on attachment and ingestion can now be determined using CD46-specific antibodies coupled to different sized beads. Nevertheless, it is clear from these studies that CD46 cross-linking induces a dramatic change in the mechanism by which the receptor is internalized and that this change correlates with the down-regulation of CD46.

The studies described here show that CD46 can be internalized by two alternative mechanisms, utilizing either clathrin-coated pits or macropinocytosis. The different fate of CD46 internalized via each pathway is likely to influence antigen presentation of CD46-internalized ligands, complement regulation (by affecting CD46 expression at the cell surface), and signal transduction through CD46. Furthermore, these investigations suggest for the first time a role for CD46 in mediating the engulfment of pathogens or complement-bound particles.

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**REFERENCES**

1. Santoro, F., Kennedy, P. E., Locatelli, G., Malnati, M. S., Berger, E. A., and Lusso, P. (1999) Cell 99, 817–827.
2. Oglesby, T. J., Allen, C. J., Liszewski, M. K., White, D. J., and Atkinson, J. P. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 2489–2493.
3. Kallstrom, H., Liszewski, M. K., Atkinson, J. P., and Jonsson, A. B. (1997) Mol. Microbiol. 23, 639–647.
4. Oglesby, T. J., Allen, C. J., Liszewski, M. K., White, D. J., and Atkinson, J. P. (1992) J. Exp. Med. 175, 1547–1551.
5. Doris, R. E., Marcel, A., Chopra, A., and Richardson, C. D. (1993) Cell 75, 295–305.
6. Naniche, D., Varior-Krishnan, G., Cervoni, F., Wild, T. F., Rossi, B., Rabourdin-Come, C., and Gerlier, D. (1993) J. Virol. 67, 6025–6032.
7. Zaffran, Y., Destaing, O., Roux, A., Ory, S., Nieu, T., Jurdic, P., Rabourdin-Come, C., and Astier, A. L. (2001) J. Immunol. 167, 6780–6785.
8. Astier, A., Trescol-Biemont, M. C., Arcoar, O., Lamouille, B., and Rabourdin-Come, C. (2000) J. Immunol. 164, 6901–6906.
9. Marie, J. C., Kehren, J., Trescol-Biemont, M. C., Eivalash, A., Valentin, H., Walser, T., Tedone, R., Loveland, B., Nicolas, J. F., Rabourdin-Come, C., and Horvat, B. (2001) J. Immunol. 164, 69–79.
10. Marie, J. C., Astier, A. L., Rivailler, P., Rabourdin-Come, C., Wild, T. F., and Horvat, B. (2002) Nat. Immunol. 3, 659–666.
11. Kemper, C., Chan, A. C., Green, J. M., Brott, K. A., Murphy, K. M., and Atkinson, J. P. (2000) Nature 421, 388–392.
12. Karp, C. L., Liszewski, M. K., Atkinson, J. P., and Jonsson, A. B. (1997) J. Exp. Med. 180, 1827–1831.
13. Hirano, A., Yang, Z., Katayama, Y., Korte-Sarfaty, J., and Wong, T. C. (1999) J. Virology 73, 4776–4785.
14. Katayama, Y., Hirano, A., and Wong, T. C. (2000) J. Virol. 74, 1252–1257.
15. Kurita-Taniguchi, M., Fukui, A., Hazeki, K., Hirano, A., Tsuji, S., Matsumoto, M., Watanabe, M., Ueda, S., and Seya, T. (2000) J. Immunol. 165, 5143–5152.
16. Gerlier, D., Trescol-Biemont, M. C., Varior-Krishnan, G., Naniche, D., Fugier-Vivar, I., and Rabourdin-Come, C. (1994) Cell Biol. Int. 18, 315–320.
17. Rivailler, P., Trescol-Biemont, M. C., Gimenez, C., Rabourdin-Come, C., and Horvat, B. (1998) Eur. J. Immunol. 28, 1301–1314.
18. Cardoso, A. I., Beavarger, P., Gerlier, D., Wild, T. F., and Rabourdin-Come, C. (1995) Virology 212, 255–258.
19. Gerlier, D., Trescol-Biemont, M. C., Varior-Krishnan, G., Naniche, D., Fugier-

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Ligand Binding Determines Whether CD46 Is Internalized by Clathrin-coated Pits or Macropinocytosis
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