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NOTES

Coxsackievirus B3 Adapted to Growth in RD Cells Binds to Decay-Accelerating Factor (CD55)

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A coxsackievirus B3 (CB3) isolate adapted to growth in RD cells shows an alteration in cell tropism as a result of its capacity to bind a 70-kDa cell surface molecule expressed on these cells. We now show that this molecule is the complement regulatory protein, decay-accelerating factor (DAF) (CD55). Anti-DAF antibodies prevented CB3 attachment to the cell surface. Radiolabeled CB3 adapted to growth in RD cells bound to CHO cells transfected with human DAF, whereas CB3 (strain Nancy), the parental strain, did not bind to DAF transfectants. These results indicate that growth of CB3 in RD cells selected for a virus strain that uses DAF for cell surface attachment.

Coxsackievirus B3 (CB3) interacts with at least two different HeLa cell surface proteins. The prototype Nancy strain of coxsackievirus B3 (CB3-N) forms a detergent-stable complex with a 49 kDa protein on the HeLa cell surface (18) and identifies a protein of similar size in virus overlay blots (14). In contrast, CB3 adapted to growth in RD cells (CB3-RD) shows an altered receptor specificity (22) and binds a 60- to 70-kDa protein in virus blot experiments (14). The monoclonal antibody (MAb) RmcA, which protects HeLa cells from CB3-N and RD cells from CB3-RD infection (9), similarly recognizes a 60- to 70-kDa protein (20). Decay-accelerating factor (DAF) (CD55), a 70-kDa complement regulatory protein, was recently shown to be the receptor for a number of echoviruses, including echovirus 6 (2, 23). Because RmcA had previously been noted to protect cells from infection by echovirus 6 as well as by CB3 (9), we investigated whether the 60- to 70-kDa protein recognized by RmcA is DAF and whether CB3-RD binds DAF on the cell surface.

RmcA recognizes DAF. HeLa cells were iodinated by the glucose oxidase-lactoperoxidase method (15). Labeled cells were extracted with 1% Nonidet P-40 (NP-40), and immunoprecipitation was performed as previously described (5) with RmcA (9) and with the anti-DAF MAb IA10 (16) (provided by Victor Nussenzweig, New York University). Both antibodies precipitated proteins migrating at approximately 75 kDa under reducing conditions (Fig. 1). In nonreducing immunoblots, RmcA identified a broad protein band migrating between 60 and 70 kDa in HeLa cell extracts, as previously reported (20), and a less intense 60-kDa protein in extracts of RD cells (data not shown). As expected, no reactive proteins were identified in extracts of murine L cells. Identical patterns were seen in immunoblots with the anti-DAF antibody IF7.

As determined by indirect immunofluorescent flow cytometry, RmcA and the anti-DAF MAb IF7 (2) both reacted with CHO cells transfected with human DAF cDNA (17), whereas neither antibody reacted with mock-transfected CHO cells (Fig. 2). These results indicate that RmcA recognizes DAF.

CB3-RD binds to CHO cells expressing human DAF. CB3-N and CB3-RD were radiolabeled by growth in [^{35}S]methionine and purified by sucrose gradient centrifugation as previously described for echovirus 1 (6). Monolayers of DAF-transfected or mock-transfected CHO cells, or HeLa cell monolayers, were incubated with the anti-DAF MAb IF7 or with a control MAb, and then the antibodies were removed and the monolayers were incubated with radiolabeled viruses. Although no virus binding to mock-transfectants was detected, radiolabeled CB3-RD bound to DAF-transfected CHO cells (Fig. 3A) and binding was prevented by preincubation with IF7. In contrast, CB3-N, the parent strain, did not bind to DAF transfectants (Fig. 3B). In accord with previous experiments using RmcA (9,

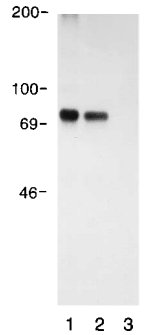

FIG. 1. Immunoprecipitation with RmcA and the anti-DAF MAb IA10. HeLa cells were iodinated by the glucose oxidase-lactoperoxidase method, extracts were prepared in buffer containing 1% NP-40, MAbs were added, and immunoprecipitation was performed with protein A-Sepharose beads precoated with rabbit anti-mouse immunoglobulin. Lane 1, IA10; lane 2, RmcA; lane 3, control MAb MOPC 195. Molecular masses (in kilodaltons) are indicated on the left.

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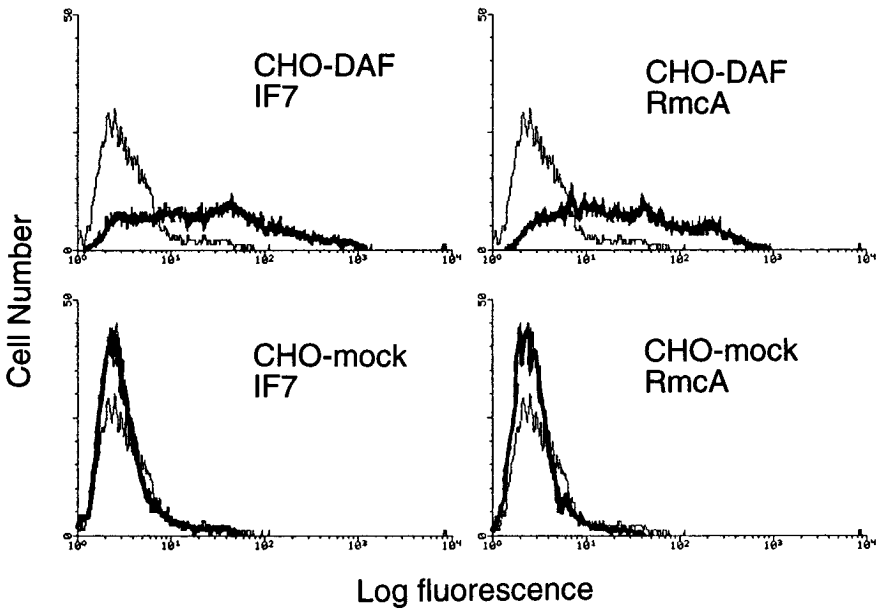

FIG. 2. Immunofluorescence analysis of CHO cell transfectants. Cells were incubated with the murine anti-DAF MAb IF7 (left panels) or with RmcA (right panels) and then with fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin; the cells were then analyzed in a fluorescence-activated cell sorter. Staining of DAF transfected cells (top panels) and of mock-transfected cells (bottom panels) is shown. Staining with the irrelevant murine MAb 5E2B4 (which recognizes the intracellular protein Tau; provided by Ken Kosik, Brigham and Women's Hospital) is shown at the left in each panel (fine line) and is partially obscured by the overlap with IF7 and RmcA staining of the mock-transfectant.

13), anti-DAF MAbs inhibited the attachment of both virus strains to HeLa cells.

CB3-RD did not cause cytopathic changes in DAF-transfected CHO cells, and little or no progeny virus could be detected. Similar results were recently reported for echovirus 7 (23). Thus, virus replication in these rodent cells is subject to some postattachment block.

DAF SCR2 is essential for virus attachment. DAF is a 70-kDa glycoprotein with four independently folded short consensus repeat (SCR) domains typical of complement regulatory proteins (7, 19). We measured the attachment of radiolabeled virus to CHO cells transfected with cDNAs from which individual SCR domains were deleted (8). Indirect immunofluorescence with anti-DAF MAbs confirmed that all the deletion mutants were expressed at equal levels on transfected cells, although wild-type expression was significantly higher (Fig. 4B). CB3-RD did not bind to transfectants expressing DAF from which SCR2 had been deleted, although the deletion of other SCRs did not prevent virus binding (Fig. 4A). These data indicate that SCR2 is essential for virus attachment and may contain the virus-binding site. Anti-DAF MAbs that recognize epitopes within SCR3, as well as those that recognize SCR2, blocked virus attachment (data not shown). This may be due to steric effects or may indicate that the virus interacts with portions of SCR3 as well as SCR2.

Discussion. Passage of CB3-N in RD cells resulted in the isolation of a virus strain with altered tropism (22). CB3-RD is capable of attaching to the RD cell surface, and as a consequence, it shows an approximately 10^6-fold increased plaquing efficiency on RD cells compared with that of the parent strain (22). The results presented here indicate that the molecular basis of this altered tropism is the acquired capacity of CB3-RD to bind to DAF, a protein that is abundant on RD cells. A second phenotypic change, the capacity of CB3-RD to

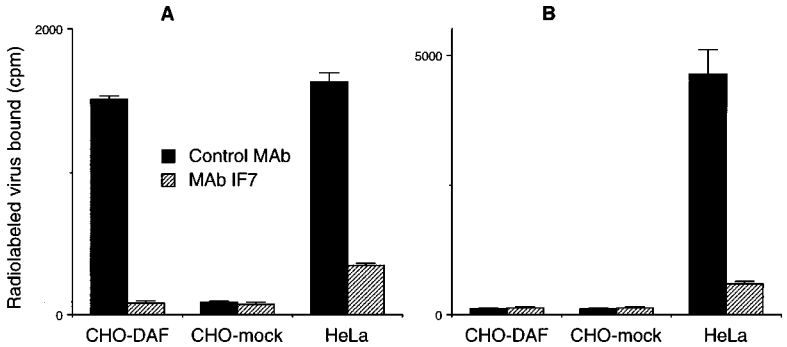

FIG. 3. CB3-RD and CB3-N attachment to DAF-transfected cells. Confluent monolayers of CHO cells transfected with human DAF cDNA, CHO cells mock-transfected with vector alone, or HeLa cells were incubated with the anti-DAF MAb IF7 or a control antibody (23A-5-21S, murine anti-H-2 K^d, obtained from the American Type Culture Collection, Rockville, Md.) and then exposed to radiolabeled CB3-RD (19,000 cpm added) (A) or prototype CB3-N (27,000 cpm added) (B) for 1 h at room temperature. The monolayers were washed extensively and then dissolved for the determination of cell-bound radioactivity. The graph shows mean virus bound (in counts per minute) ± standard deviations for triplicate samples.

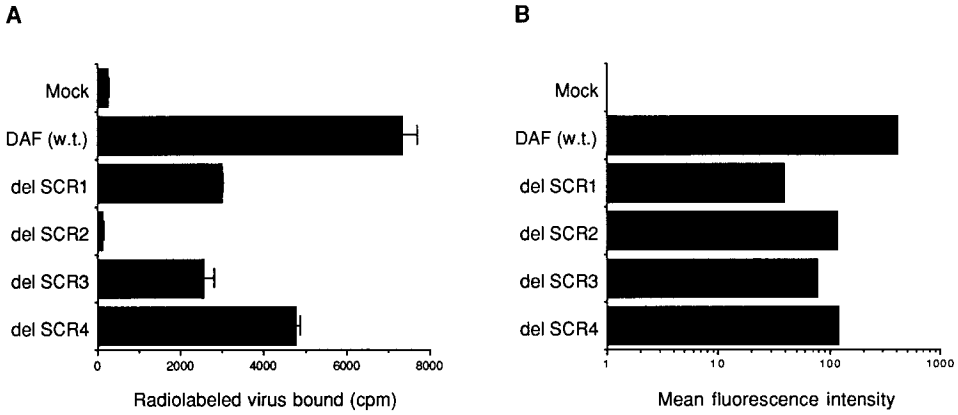

FIG. 4. CB3-RD attachment to SCR deletion mutants. (A) Virus binding. Confluent monolayers of CHO cells transfected with cDNA encoding wild-type DAF or DAF from which individual SCR domains were deleted, or monolayers of CHO cells mock-transfected with the expression vector alone, were incubated with radiolabeled virus and then washed extensively and dissolved for scintillation counting. The graph shows mean virus bound (in counts per minute) ± standard deviations for triplicate samples. (B) Cell surface expression of DAF on transfected cells determined by flow cytometry with anti-DAF antibodies. Transfected CHO cells were dispersed with 1 mM EDTA and then stained with the anti-DAF MAb IA10, followed by fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin. Because IA10 recognizes an epitope within SCR1 (8), expression of the SCR1 deletion mutant was measured with MAb 8D11 (8), which recognizes SCR4 (provided by Wendell Rosse, Duke University). IA10 and 8D11 stained wild-type DAF transfectants with equal intensity. Mean cell fluorescence was measured on a Becton-Dickinson FACScan, using Lysys 2 software. Values are expressed relative to the fluorescence of cells stained with the irrelevant MAb 5E2B4, which was given the arbitrary value of 1. DAF (w.t.), wild-type DAF; del SCR1, DAF from which the SCR1 domain has been deleted.

agglutinate erythrocytes (22), may be due to an interaction with DAF present on human erythrocytes.

CB3-N binds to an unidentified 49-kDa protein present on HeLa but not on RD cells (reference 18 and unpublished data). In contrast to CB3-RD, CB3-N did not bind to DAF transfectants. Nonetheless, anti-DAF antibodies prevent attachment of CB3-N to HeLa cells (9, 13) (Fig. 3). This suggests that CB3-N contacts both DAF and the 49-kDa protein on the cell surface, although it may have little or no affinity for DAF itself. Experiments are in progress to determine whether DAF and the 49-kDa protein are associated on the cell surface.

It had previously been noted that several virus receptors belong to the immunoglobulin gene superfamily (24). It appears now that viruses are equally likely to bind to integrins (3) or to complement regulatory proteins. Epstein-Barr virus interacts with human complement receptor 2 (11), a protein composed of 15 SCR domains, and measles virus binds to membrane cofactor protein (10, 21), which is composed of 4 SCR domains. Both DAF and membrane cofactor protein function in protecting cells from lysis by autologous complement. Perturbations of complement regulation resulting from virus-receptor interactions could play a role in viral pathogenesis.

Coxsackieviruses of group B cause febrile illnesses in humans and are the viruses most commonly associated with infectious myocarditis (12). In this regard, it is of interest that DAF is expressed widely on endothelium (1) and is present on cardiac myocytes (25). In ongoing experiments, we find that many clinical coxsackievirus isolates are inhibited by anti-DAF antibodies and that some virus strains never grown in RD cells bind directly to isolated DAF or to DAF transfectants (4). Further work will be required to determine how interactions with DAF or with the 49-kDa putative receptor contribute to virus tropism in vivo.

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