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HUMAN CORONAVIRUS OC43 INTERACTS WITH MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I MOLECULES AT THE CELL SURFACE TO ESTABLISH INFECTION

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ABSTRACT

Human coronaviruses have been associated with common colds, diarrhea and enterocolitis, and have been implicated in multiple sclerosis. HLA class I molecules may play a critical role as receptor for OC43 because monoclonal antibody (mAb)W6/32 to HLA-A, -B and -C specificities completely blocks infectivity in human rhabdomyosarcoma (RD) cells. The role of HLA class 1 antigen as the virus receptor was examined using HLA-A3.1 stably transfected human plasma cells and untransfected HMY.C1R cells which do not express HLA-A and -B molecules. When the cells ($5x10^6$) were infected at a multiplicity of one, the HLA.A3 transfected cells produced 10^8 PFU of virus whereas no replication occurred in the HMY.C1R cells mAb W6/32 reduced the virus yield by 99.9% Cell membranes from HMY.C1R, HMY.A3 cells and chicken erythrocytes were biotinylated as live cells. Immunoprecipitation with polyclonal antiviral antibody to detect binding of biotinylated cell membranes to virus revealed that biotinylated HMY.A3 membranes co-precipitated with virus-antibody complexes when the immunoprecipitates were electrophoresed on SDS-PAGE gel, electroblotted and stained with Avidin-horseradish peroxidase. The results provide direct evidence that OC43 virus can recognize HLA class I as receptor on the cell surface.

Key words: HLA Class I receptor - OC43 virus Interaction

Abbreviations used: RD, human rhabdomyosarcoma cells; HLA, human leucocyte antigen; HMY.A3.1, human plasmacytoma cells transfected with HLA-A3 gene, HMY.C1R human plasmacytoma cells that lack HLA-A and B molecules on the cell surface; MHC-I, major histocompatibility complex class I; Fbs, fetal bovine serum; pfu, virus plaque forming units; Seph Pro A, protein A sepharose CL-4B; 2-me, 2-mercaptoethanol; mAb, monoclonal antibody; Avidin-HRP, avidin conjugated to horseradish peroxidase; 4CN, 4-chloro-1 naphthol; CEA, carcinoembryonic antigen.

INTRODUCTION

Human coronaviruses have been associated with common colds, diarrhea and enterocolitis, and possibly multiple sclerosis (1-4). The prototypes, 229e and OC43 are enveloped, positive stranded, non-segmented RNA viruses. The RNA of approximately 31kb, contains a 5' cap and a 3' poly(A) tail and is infectious. Genomic and eight subgenomic RNAs serve as templates for translation of the proteins. The enormous size of the RNA suggests a unique mechanism of replication that must control for the possible deleterious effects of errors in RNA synthesis (5). OC43 virions contain two surface peplomers. The spike (S) is 190kd in size and is responsible for binding to 9-0-acetyl neuraminic acid residues on the surface of erythrocytes while the hemagglutinin/esterase (HE) peplomer has acetylesterase activity which removes this carbohydrate residue (6).

The steps involved in binding of OC43 virus to its receptor and subsequent entry and initiation of infection make up the set of early events that determine what cells will become infected. A preliminary study of entry of OC43 into cells has revealed that HLA class I molecules apparently play a critical role because monoclonal antibody W6/32 to HLA A, B and C specificities completely blocked infectivity in human rhabdomyosarcoma (RD) cells (7). HLA class I molecules are expressed on all membranes of nucleated cells in the human body but are present in low amounts in neuron and glial cells. The HLA class I molecule consists of two chains, beta 2-microglobulin (mw 12,000) and heavy chain glycoprotein (mw 44,000). The structure of several HLA B and A molecules has been determined by X-ray crystallography and reconstitution of the heterodimer requires binding of an antigenic peptide (9-11 mer) in the antigen binding cleft (8).

A study of receptor binding and entry of OC43 virus into HLA transfected human plasmacytoma cells was undertaken to further determine the role of this molecule as the cellular receptor for the virus. The HLA-A3 antigen was selected as one of the set of major histocompatibility class I molecules expressed on human cells. As yet there is no evidence associating specific alleles with receptor activity.

MATERIALS AND METHODS

Viruses and cells

Human coronaviruses OC43, obtained from G. Gerna (2) and 229e from ATCC were propagated and plaque formation assayed in MRC-5, human diploid lung cells (ViroMed,

Minnetonka, MN 55343). In immunoprecipitation experiments, supernatant virus was harvested from Human Rhabdomyosarcoma (RD) and concentrated 100-fold by ultracentrifugation (7).

The human plasma cell line HMY.C1R which does not express HLA-A and B molecules as determined by HLA typing was transfected by calcium phosphate precipitation with a plasmid clone containing the HLA-A3.1 (9). The transfected cells were assayed for surface expression of A3 molecules by indirect immunofluorescence using the HLA-A3-specific monoclonal antibody, GAP.A3 (10). HMY.C1R and HMY.A3.1 were received from Dr. F. Ennis, University of Massachusetts Medical Center, Wooster, MA. Daudi cells were obtained from Dr. M-L. Hammarskjöld in the Department of Microbiology, SUNY, Buffalo. The cells were cultivated in RPMI 1640 medium with 10% heat-inactivated fetal bovine. Surface expression of HLA-A3 was confirmed by HLA Typing by Dr. T. Shanahan, Director, Histocompatibility Laboratory, Erie County Medical Center, Buffalo, NY.

Antibodies

Rabbit anti-OC43 polyclonal antibody was prepared by immunization with gradient purified virus from infected suckling mouse brain (7). W6/321, mouse monoclonal anti-HLA class I-ABC was obtained from C-six Diagnostics, Inc., Mequon, WI 53092.

Chemicals

NHS-d-Biotin (N-hydroxysuccinimidobiotin, MW 341.4) and Triton X-100 were obtained from Sigma, St. Louis, MO 63178. Protein A Sepharose CL-4B (SephProA) from Pharmacia LKB, Uppsala, Sweden, Avidin Horseradish Peroxidase and 4-chloro-1-naphthol substrate developer were purchased from BioRad, Hercules, CA 94547.

Labeling of cells with biotin

The two step procedure for biotin labeling of cell surface proteins was followed (11). HMY and HMY.A3 cells (10^8) were washed 3 times in ice-cold PBS (0.1M, pH 7.2) and incubated at 0°C for 10 min with 2ml biotin solution (0.2mg NHS-d-biotin/mlPBS). Following removal of residual biotin by five washes with PBS containing 0.1% bovine serum albumin, cells were treated with lysis buffer (20nM Tris-HCL, pH 8.0, 1mM Na_2-EDTA, 150mM NaCl, 1mM phenylmethylsulfonyl fluoride, 1% Ionidet P-40 and 0.02% NaN_3). After twenty strokes with a Dounce homogenizer and 30 min sonication (400 watts), the insoluble material was removed by centrifugation (100,000g, 1h, 4°C). To biotin label

chicken erythrocytes (ChRbc), cells were washed 3 times in PBS and 0.2ml of packed cells was incubated with 2ml of biotin at 0°C for 10 min and washed 5 times with PBS containing 1% bovine serum albumin. Ten ml of hypotonic buffer (7.5mM sodium phosphate pH 7.5, 1mM EDTA; 1mM phenylmethyl sulfonyl fluoride) were added and ghosts and hemoglobin were separated by centrifugation (50,000g, 30 min, 4°C). After washing once, ChRbc ghosts were treated with lysis buffer to solubilize proteins. Solubilized proteins were used for analysis of virus binding by immunoprecipitation and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

SDS-PAGE, Western transfer and blotting

Proteins were run on 10% acrylamide gels (12) with subsequent electrotransfer (TransPhor, Hoeffer, San Francisco, CA 94107) for 30 min to Immobilon-P membranes (Millipore, Bedford, MA 01730) (13). Reactive sites on the membranes were blocked by incubation with TBS (0.01M Tris buffer pH 7.5, 0.15M NaCl) containing 5% bovine serum albumin (BSA) at 37°C for 30 min. Biotinylated proteins were detected by incubation with Avidin Horseradish peroxidase (1:1000 dilution in TBS containing 0.1% BSA).

Immunoprecipitation of Virus-Receptor Complexes

As described (14), biotin-labeled cell surface proteins from HMY.C1R, HMY.A3, and ChRbc were precleared by adding normal rabbit serum, 2ul/50ul of membranes, and 10ul of SephProA (prepared as a 1:1 slurry in Tris/saline/azide (TSA) solution: 0.01M Tris.Cl.pH 8.0, 0.14M NaCl, .025% NaN_3) let stand 12-18 hr at 4°C, centrifuged 10 min at 1000xg and supernatant removed. Then 50-200μl virus (100x supernatant from infected RD cells) diluted in TBS and 10μl rabbit anti-OC43 antibody was added and the mixture incubated 4h at 4°C with vigorous shaking. Then 25μl sephProA was added and reincubated 4h at 4°C with vigorous shaking. Unbound material was removed by centrifugation at 1000xg for 10 min and the immunoprecipitate washed twice with TBS containing 0.1% Triton X-100 and once with TBS before adding 20μl of SDS/sample buffer without 2-mercaptoethanol. Samples were heated for 5 min at 100°C before loading into gel lanes for SDS/PAGE analysis and biotin blotting.

RESULTS

Effect of HLA on virus replication. The susceptibility of human coronavirus OC43 and 229e replication in RD cells to the receptor blockade effect of anti-HLA antibody was

TABLE I

Effect of HLA on Virus Replication

Virus	Cells	Virus yield (log_{10} PFU/ml)[1]			
		HLA antibody dilution[2]			virus
		1:200	1:400	1:800	control
OC43	RD	<2	<2	4.2	5.1
	HMY.A3.1	3.0	—[3]	—	6.7
	HMY.C1R	—	—	—	2.7
	Daudi	—	—	—	<2
229e	RD	5.5	5.6	5.8	5.8
	Daudi	—	—	—	<2

[1] Cells were infected at a multiplicity of 1.

[2] Cells were pretreated with mAb W6/32 for 1 hr, then mAb was removed and cells infected, washed twice and resuspended in medium containing mAb.

[3] — = not done

examined (TABLE I). HLA antibody prevented OC43 infection of RD cells but not 229e. The lack of effect of HLA antibody on 229e virus binding was not surprising since 229e is known to utilize aminopeptidase-N as cell receptor (15). Also, the infectability of Daudi (HLA-) cells by OC43 and 229e was examined. Neither virus replicated, suggesting that receptors for both viruses were absent.

Next, susceptibility of HLA-A3 transfected and HMY untransfected cells to OC43 infection was examined (Figure 1). It was found that OC43 replicated in the HLA transfected cells to a titer of 10^8 PFU when $5x10^6$ cells were infected at a multiplicity of one in triplicate. Also, the virus titer was reduced 1000-fold in the presence of anti-HLA antibody (TABLE I).

In HMY.C1R cells, virus at low levels (10^2 PFU) was recovered from the supernatant medium after washing. However, no significant progeny virus was produced.

Direct binding of OC43 to biotinylated cell surface proteins. Since HLA molecules contain only one glycosyllation site on the α chain and one on the $\beta 2$-

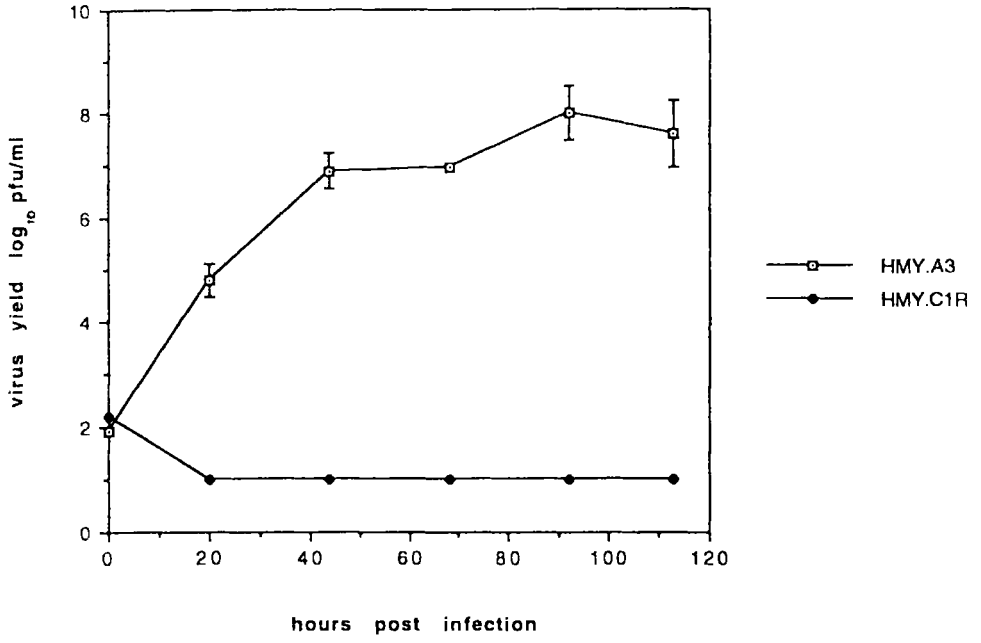

FIGURE 1

Infectivity of OC43 virus for cells expressing HLA class I molecules. Cells were infected at a multiplicity of 1, washed twice and incubated in RPMI 1640 medium with 0.1% Fbs. Results for virus titer ($\bar{\chi} \pm$ SE) are from triplicate experiments. Cell viability was determined daily; variation was within 20% of uninfected controls.

microglobulin and normally cycle from within the cell where they bind endogenous antigen peptides and migrate to the cell surface where they present the peptides for recognition by CD8 cytotoxic T cells, the location of the site at which OC43 binding to HLA molecules occurs needed to be determined. Direct virus binding to cell surface proteins was examined by selectively labeling live-cell surface proteins with a biotin label and immunoprecipitation of virus-receptor complexes with anti-OC43 antibody. Equal numbers (10^8) of HMY.A3.1, HMY.C1R cells and ChRbc were biotin labeled with N-hydroxysuccimidobiotin. The membranes for use in immunoprecipitation were precleared with normal rabbit serum and Sepharose protein A. Various amounts (50-200μl) of cell-free virus and rabbit anti-OC43 antibody were added and the mixtures were incubated for 4 hr at 4°C. Sepharose protein A was then added and the mixtures were reincubated for 4 hr at 4°C. The resulting immunoprecipitates were washed three times, dissolved in sample buffer without reducing agent, run on SDS/PAGE, electroblotted and reacted with avidin HRP to detect the amounts

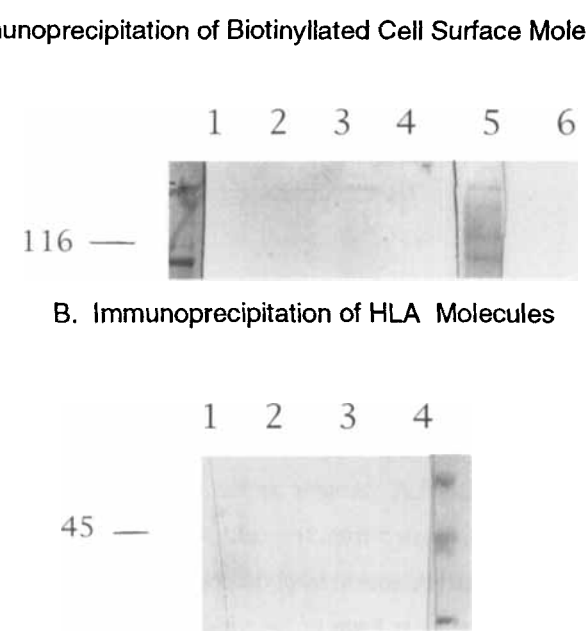

FIGURE 2

Immunoprecipitation of virus-bound HLA class I molecules.

A. Biotinylated cell membrane proteins were mixed with virus and anti-viral antibody (10μl). Immune complexes were precipitated by Seph Pro A (25μl), dissociated in sample buffer without 2-me, electrophoresed on 10% SDS-PAGE, blotted to Immobilon (Millipore), reacted with avidin HRP, and developed with 4 CN. On the left, proteins of 97kd and 116kd (arrow) are markers. Lanes 1-3 show a high molecular weight (400kD) band precipitated from HMY.A3 cell lysates. Lane 1 50μl, Lane 2 100μl and Lane 3 200μl of virus, lane 4 no virus. Lane 6 200μl of virus and HMY.C1R cell lysate, Lane 5 virus only; reaction obtained with OC43 antibody and goat anti-rabbit HRP. Viral proteins gp 112 (S) and gp 130 (HE) and a high molecular weight aggregate are visible.

B. Detection of HLA α-chain in immunoprecipitates of virus-receptor complexes from unbiotinylated RD cell membranes (50μl). Lane 1 100 μl OC43 antibody, Lane 2 50μl OC43 antibody, Lane 3 and 4 same as 1 and 2 except no virus. Right side, 66kD, 45kD (arrow) and 31kD markers.

of biotinylated cell surface proteins that were co-precipitated. In this experiment (FIGURE 2A), a high molecular weight (400,000 dalton) avidin-binding protein was found in increasing amounts in proportion to increasing virus concentration in the immunoprecipitates formed by mixing OC43 virus and HMY.A3.1 cells. The avidin-binding 400kD molecule was not observed to precipitate with HMY.C1R or ChRbc membranes (not shown). When unbiotinylated RD cell membranes were mixed with virus for immunoprecipitation of virus-

receptor complexes and the Western blot was reacted with mAb W6/32, a band at 44kD, the size of the HLA α chain, was seen (FIGURE 2B).

DISCUSSION

Haywood (15) has defined the term virus receptor to mean a host surface component that participates in virus binding and facilitates viral infection. The experiments presented here have attempted to show that OC43 utilizes HLA class I molecules expressed on the cell surface as biologically functional receptors. Identical untransfected cells lacking HLA were not infected after exposure to virus suggesting that they lack functional receptors. Direct evidence of virus binding to the HLA receptor at the cell surface was obtained using biotinylated cell surface proteins prepared from live cells to form virus-receptor complexes. Helenius et al. (16), using ^{35}S sulfanilic acid to label the cell membrane, showed that Semliki Forest virus 29S complexes (octavalent form of the viral spikes) bound to HLA and H-2 molecules could be isolated. However these complexes failed to form with plasma membranes released by detergent lysis suggesting these were weak binding forces since multimerization of virus spikes and membrane vesicles was required (9). HLA molecules are the major species and alloantigens on the cell surface responsible for self recognition. The murine MHC-I can also function as a determinant of infectivity for murine cytomegalovirus (17). Murine coronaviruses utilize a mouse specific epitope on biliary glycoprotein (CEA) as receptor (18). CEA, like HLA, is a member of the immunoglobulin gene superfamily, all members of which share a characteristic immunoglobulin fold domain having a length of about 100 amino acids, a conserved disulfide bond spanning 60 amino acids and a β pleated-sheet structure(19). Binding to HLA class I molecules, which are present in variable amounts on all nucleated cells, could explain the susceptibility of the human upper respiratory mucosa to OC43 virus but would not explain the potential neurotropism of this virus since neuronal cells are MHC class I deficient. Mechanisms such as interferon γ-induced enhancement of HLA class I molecules may account for increased susceptibility of neuronal and other cells to this virus during infection.

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