Intestinal Epithelial CD98 Directly Modulates the Innate Host Response to Enteric Bacterial Pathogens

Moiz A. Charania,^a Hamed Laroui,^a Hongchun Liu,^{c,e} Emilie Viennois,^a Saravanan Ayyadurai,^a Bo Xiao,^a Sarah A. Ingersoll,^{a,b} Daniel Kalman,^d Didier Merlin^{a,b}

Department of Biology, Center Diagnostics and Therapeutics, Georgia State University, Atlanta, Georgia, USA^a; Veterans Affairs Medical Center, Decatur, Georgia, USA^b; Division of Digestive Diseases, Emory University School of Medicine, Atlanta, Georgia, USA^c; Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, Georgia, USA^d; Department of Gastroenterology, Zhongshan Hospital, Fudan University, Shanghai, China^e

CD98 is a type II transmembrane glycoprotein whose expression increases in intestinal epithelial cells (IECs) during intestinal inflammation. Enteropathogenic *Escherichia coli* (EPEC) is a food-borne human pathogen that attaches to IECs and injects effector proteins directly into the host cells, thus provoking an inflammatory response. In the present study, we investigated CD98 and EPEC interactions *in vitro* and *ex vivo* and examined FVB wild-type (WT) and villin-CD98 transgenic mice overexpressing human CD98 in IECs (hCD98 Tg mice) and infected with *Citrobacter rodentium* as an *in vivo* model. *In vivo* studies indicated that CD98 overexpression, localized to the apical domain of colonic cells, increased the attachment of *C. rodentium* in mouse colons and resulted in increased expression of proinflammatory markers and decreased expression of anti-inflammatory markers. The proliferative markers Ki-67 and cyclin D1 were significantly increased in the colonic tissue of *C. rodentium*-infected hCD98 Tg mice compared to that of WT mice. *Ex vivo* studies correlate with the *in vivo* data. Small interfering RNA (siRNA) studies with Caco2-BBE cells showed a decrease in adherence of EPEC to Caco2 cells in which CD98 expression was knocked down. *In vitro* surface plasmon resonance (SPR) experiments showed direct binding between recombinant hCD98 and EPEC/C. *rodentium* proteins. We also demonstrated that the partial extracellular loop of hCD98 was sufficient for direct binding to EPEC/C. *rodentium*. These findings demonstrate the importance of the extracellular loop of CD98 in the innate host defense response to intestinal infection by attaching and effacing (A/E) pathogens.

Cell-cell and cell-matrix interactions play fundamental roles in creating and maintaining the polarity of intestinal epithelial cells. Cell-cell and cell-matrix interactions also contribute to the formation of an intrinsic barrier against microbial invaders. Polarized intestinal epithelial cells (IECs) contain distinct apical and basolateral membrane domains with unique protein and lipid compositions. The major epithelial permeability barriers are controlled and regulated by a cascade of events triggered by cell-cell and cell-extracellular matrix (ECM) contacts (1–3).

Specific proteins, such as tight junction proteins, participate in sealing the space between neighboring cells and promoting cell polarization, which together generate an impermeable barrier between the IECs and the bacterium-containing outside world. Because epithelial cells rest on the ECM, it is reasonable to expect specific interactions between basolateral receptors, such as integrins, and the ECM. Indeed, cell-matrix interactions are also important in maintaining intestinal barrier function. Cell-matrix interactions involving surface adhesion molecules, such as integrins, are required for normal epithelial development, and mutation or absence of these molecular adhesins can disrupt growth and/or alter epithelial cell function and cell polarity (4–6).

Pathogens, including viruses and bacteria, exploit cell-adhesion molecules to directly or indirectly disrupt epithelial polarization and tight junctions (7–10). Among pathogens, enteropathogenic *Escherichia coli* (EPEC), which infects human intestines, and *Citrobacter rodentium*, its murine equivalent, are termed attaching and effacing (A/E) pathogens because of their ability to intimately bind to the apical surface of intestinal epithelial cells (11–14). A/E is a complex multistep process that requires type III secretion machinery (15–18). The type III machinery delivers bacterial proteins and effectors, including Tir (translocated intimin receptor),

EspF, EspG, and Map—all of which have an indirect effect on tight junction protein integrity—into the cytoplasm of host intestinal epithelial cells. Interestingly, it has been demonstrated *in vitro* that intimin interacts with cells independently of Tir and binds to β 1-integrins (19), resulting in disruption of intestinal barrier and polarity functions of the intestinal epithelial layer.

During intestinal inflammation, the loss of intestinal epithelial barrier and polarity functions induces the redistribution of basolateral membrane proteins such as β 1-integrin to the apical cell surface (20). The induction of redistribution of β 1-integrin to the intestinal epithelial apical cell surface by EPEC provides additional protein-interacting partners for EPEC. Recently, we and others have shown that CD98, a cell surface protein formed by covalent linkage of CD98 heavy chain (CD98hc) with several different light chains to form amino acid transporters, also functions as a β 1-integrin regulator. Our laboratory and other researchers have also demonstrated that CD98 expression is upregulated during intestinal inflammation (21–24). In addition, it has also been

Received 6 December 2012 Accepted 27 December 2012

Published ahead of print 7 January 2013

Editor: A. J. Bäuml

Address correspondence to Moiz A. Charania, mcharania@gsu.edu.

We dedicate this article to the memory of Shanthi V. Sitaraman, a brilliant scientist, dedicated physician, passionate humanitarian, and dearest friend.

Supplemental material for this article may be found at <http://dx.doi.org/10.1128/IAI.01388-12>.

Copyright © 2013, American Society for Microbiology. All Rights Reserved.
doi:10.1128/IAI.01388-12

shown that one of the roles of CD98 is to enhance $\beta 1$ -integrin cell signaling, including prosurvival signaling (25–33). Interestingly, a recent study suggested that in EPEC, the type III effector EspZ interacts with host CD98 and facilitates host cell prosurvival signaling (34).

In the present study, we examined the potential role of epithelial CD98 during EPEC and *C. rodentium* infection, exploiting human CD98-villin transgenic (hCD98 Tg) mice and using *in vivo*, *in vitro*, and *ex vivo* approaches. By *in vivo* and *ex vivo* studies, we looked at whether CD98 overexpression in IECs has an effect on the inflammation response and attachment of EPEC and *C. rodentium* during infection, and by *in vitro* study, we looked at whether recombinant CD98 protein and specifically its extracellular domain had direct binding with both the A/E pathogens. Importantly, these studies could reveal the pathophysiological relevance of CD98 expression in the innate host response to enteric bacterial pathogens.

MATERIALS AND METHODS

Experimental animals. Six- to 8-week-old sex-matched wild-type (WT) and hCD98 Tg (35) FVB mice were used in all experiments. All mice were group housed in standard cages under a controlled temperature and photoperiod (12-h/12-h light/dark cycle). All procedures using mice were in accordance with Emory University Institutional Animal Care and Georgia State University Department of Animal Resources regulations.

Bacterial strains. WT enteropathogenic *E. coli* (EPEC) strain E2348/69 was used in *in vitro* and *ex vivo* studies, while a nonpathogenic EPEC Δeae mutant was used in the *in vitro* study. WT *Citrobacter rodentium* ATCC 51116 was used in *in vitro* and *in vivo* studies. Both bacterial strains were grown and maintained in Luria-Bertani (LB) medium (Fisher Scientific, Fair Lawn, NJ) at 37°C.

Cell culture. Caco2-BBE cells were grown in Dulbecco's modified Eagle's medium (Life Technologies, Carlsbad, CA) with 10% fetal bovine serum (FBS; Atlanta Biologicals, Lawrenceville, GA). Cells were grown at 37°C with 5% CO₂ and 90% humidity.

***In vivo* infection.** For infecting WT and CD98 Tg mice with *C. rodentium*, food and drinking water were replaced with 20% sucrose containing *C. rodentium* (3.3×10^7 CFU/ml) in distilled water overnight as previously described (36). Control groups were given normal drinking water. Seven days after infection, mice were sacrificed and colons were removed for further study.

***Ex vivo* infection.** Colonic tissues were cultured in RPMI 1640 medium (Cellgro, Manassas, VA) with FBS as described previously (36). Briefly, 5- to 10-mm colon tissues of WT and CD98 Tg mice were placed on sterile foam squares in 6-well culture plates with the mucosal surface facing upwards. The wells were flooded with RPMI 1640 medium with FBS. Tissues were infected with *C. rodentium* (grown overnight in LB medium) and incubated at 37°C for 6 h with 5% CO₂. The culture medium was changed every 2 h to maintain pH and nutrient levels without reinoculating the bacteria.

Caco2-BBE cell transfection by CD98 siRNA and infection by EPEC. Caco2-BBE cells were cultured in 6-well plastic plates until reaching 50 to 60% confluence and transfected with 150 ng CD98 small interfering RNA (siRNA) (Santa Cruz Biotechnology; sc-35033) or 150 ng of Stealth interfering RNA (RNAi) (negative siRNA control; Invitrogen; 12935-400) using Lipofectamine 2000 (Invitrogen) and Opti-MEM1 reduced serum medium (Invitrogen) according to the manufacturer's instructions. Opti-MEM1 medium with 10% FBS was added to each well after 5 h, and cells were incubated at 37°C with 5% CO₂ overnight.

Seven hundred fifty microliters of EPEC grown in LB broth overnight was inoculated in 10 ml serum-free Dulbecco modified Eagle medium (DMEM) with 0.5% mannose and incubated for approximately 3 h at 37°C on a shaker to an optical density at 600 nm (OD₆₀₀) of 0.4. Transfected cells were washed with sterile phosphate-buffered saline (PBS)

twice and infected with EPEC at a multiplicity of infection of 100 for 3 h at 37°C. Nonadherent bacteria were removed by washing the cells with PBS three times. Caco2-BBE cells with the adherent bacteria were collected in PBS, serially diluted and plated on MacConkey agar (BD, Franklin Lakes, NJ), and incubated at 37°C overnight. Pink colonies of EPEC were counted, and CFU per ml of cell lysate were calculated. The remaining Caco2-BBE cells were centrifuged, and the pellet was resuspended in RLT buffer with beta-mercaptoethanol (RNeasy minikit; Qiagen, Valencia, CA) for RNA extraction according to the manufacturer's instruction.

***C. rodentium* colony counts.** Tissue samples from the colon were homogenized and serially diluted in sterile PBS, plated on MacConkey agar (BD) plates, and incubated at 37°C overnight. Pink colonies of *C. rodentium* were counted, and CFU per mg of tissue were calculated.

RNA extraction and real-time RT-PCR. Total RNA was extracted from colonic tissue of mice or Caco2-BBE cells with the RNeasy minikit (Qiagen) according to the manufacturer's instructions. Yield and quality of RNA were verified with a Synergy 2 plate reader (BioTek, Winooski, VT). cDNA was generated from the total RNAs isolated above using the Maxima first-strand cDNA synthesis kit (Fermentas, Glen Burnie, MD) according to the manufacturer's instructions. Expression of the total RNA was quantified by real-time reverse transcription-PCR (RT-PCR) using Maxima SYBR green/ROX (6-carboxyl-X-rhodamine) quantitative PCR (qPCR) Master Mix (Fermentas). 36B4 was used as a housekeeping gene for RNA extracted from mouse colon, while glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a housekeeping gene for RNA extracted from Caco2-BBE cells. Fold induction was calculated using the threshold cycle (C_T) method as follows: $\Delta\Delta C_T = (C_{T\text{target}} - C_{T\text{housekeeping}})_{\text{group 1}} - (C_{T\text{target}} - C_{T\text{housekeeping}})_{\text{group 2}}$. The final data were derived from $2^{-\Delta\Delta C_T}$. Sequences of all the primers used for real-time RT-PCR are given in Table 1.

Protein extraction and Western blot analysis. Colonic mucosal tissues from control and *C. rodentium*-infected WT and Tg mice overexpressing CD98 were homogenized in radioimmunoprecipitation assay buffer (150 mM NaCl, 0.5% sodium deoxycholate, 50 mM Tris-HCl, pH 8, 0.1% SDS, 0.1% Nonidet P-40) supplemented with protease inhibitors (Roche Diagnostics, Indianapolis, IN) on ice. The homogenates were centrifuged at 12,000 rpm for 10 min at 4°C. Total cell lysates (50 μ g/lane) were resolved on 7.5% or 4 to 15% gradient polyacrylamide gels (Bio-Rad, Hercules, CA) and transferred to nitrocellulose membranes (Bio-Rad). Membranes were then probed with primary antibodies: CD98, cyclin D1 (Santa Cruz Biotechnology Inc., Santa Cruz, CA; sc-9160 and sc-753, respectively), and β -actin (Cell Signaling Technology, Danvers, MA; catalog no. 3700). After washes, membranes were incubated with appropriate horseradish peroxidase-conjugated secondary antibodies (GE Healthcare, Buckinghamshire, United Kingdom), and blots were detected using the enhanced chemiluminescence detection kit (GE Healthcare). Recombinant proteins (full-length CD98 and extracellular CD98 [Novus Biologicals, Littleton, CO]) were resolved by 4 to 15% SDS-PAGE and transferred to polyvinylidene difluoride membranes (Bio-Rad). As all recombinant proteins were glutathione S-transferase (GST) tagged, membranes were probed for 1 h at room temperature with anti-human GST tag antibody (Cell Signaling Technology).

H&E staining. Colonic sections were fixed in 10% buffered formalin (EMD Millipore, Billerica, MA) and embedded in paraffin. Five-micrometer sections were stained with hematoxylin and eosin (H&E). Photomicrographs were taken using an Olympus microscope and D-26 color camera at $\times 20$ magnification.

Ki-67 and Ly6g staining. Five-micrometer paraffin-embedded tissue sections were deparaffinized in xylene and rehydrated using an ethanol gradient. Tissue sections were incubated with 3% hydrogen peroxide in PBS for 30 min at room temperature. Epitope retrieval was performed by treating the tissues with 10 mM sodium citrate buffer (pH 6.0) with 0.05% Tween 20 at 100°C for 10 min in a pressure cooker. For Ki-67 antibody, sections were blocked with normal goat serum from the Vectastain ABC kit in Tris-buffered saline (TBS) for 1 h at room temperature and then incubated with rabbit polyclonal

TABLE 1 Primers used in this study

Primer name	Sequence	Description
KC-F	5'-TTGTGCGAAAAGAAGTGCAG-3'	Keratinocyte-derived chemokine gene RT-PCR forward primer
KC-R	5'-TACAAACACAGCCTCCCACA-3'	Keratinocyte-derived chemokine gene RT-PCR reverse primer
IL-6F	5'-ACAAGTCGGAGGCTTAATTACACAT-3'	Interleukin-6 gene RT-PCR forward primer
IL-6R	5'-TTGCCATTGCACAACCTCTTTTC-3'	Interleukin-6 gene RT-PCR reverse primer
IL-17aF	5'-GGTTGCCAAGCCTTATCGGA-3'	Interleukin-17a gene RT-PCR forward primer
IL-17aR	5'-ACCTGCTCCACTGCCTTGCT-3'	Interleukin-17a gene RT-PCR reverse primer
TNF- α F	5'-AGGCTGCCCCGACTACGT-3'	Tumor necrosis factor gene RT-PCR forward primer
TNF- α R	5'-GACTTTCTCCTGGTATGAGATAGCAAA-3'	Tumor necrosis factor gene RT-PCR reverse primer
IFN- γ F	5'-CAGCAACAGCAAGGCGAAA-3'	Gamma interferon gene RT-PCR forward primer
IFN- γ R	5'-CTGGACCTGTGGGTGTGAC-3'	Gamma interferon gene RT-PCR reverse primer
IL-10F	5'-GGTTGCCAAGCCTTATCGGA-3'	Interleukin-10 gene RT-PCR forward primer
IL-10R	5'-CTTCTCACCCAGGGAATTCA-3'	Interleukin-10 gene RT-PCR reverse primer
TGF- β F	5'-TGCGCTTGACAGAGATTAATA-3'	TGF- β gene RT-PCR forward primer
TGF- β R	5'-AGACAGCCACTCAGGCGTAT-3'	TGF- β gene RT-PCR reverse primer
Ccd1-F	5'-TGCGCTTGACAGAGATTAATA-3'	Cyclin D1 gene RT-PCR forward primer
Ccd1-R	5'-AGACAGCCACTCAGGCGTAT-3'	Cyclin D1 gene RT-PCR reverse primer
36B4-F	5'-TCCAGGCTTTGGGCATCA-3'	36B4 gene RT-PCR forward primer
36B4-R	5'-CTTTATCAGCTGCACATCACTCAGA-3'	36B4 gene RT-PCR reverse primer
GAPDH-F	5'-ACCACAGTCCATGCCATCAC-3'	Glyceraldehyde-3-phosphate dehydrogenase gene RT-PCR forward primer
GAPDH-R	5'-TCCACCACCCTGTTGCTGTA-3'	Glyceraldehyde-3-phosphate dehydrogenase gene RT-PCR reverse primer

anti-Ki-67 (1:50 dilution) (Vector Laboratories, Burlingame, CA) in TBS for 1 h at 37°C. For Ly6g staining, sections were blocked with 10% normal goat serum with 1% bovine serum albumin (BSA) in TBS for 2 h at room temperature followed by incubation with rat monoclonal anti-Ly6g antibody (1:500 dilution) (Abcam, Cambridge, MA; ab25377) in TBS with 1% BSA at 4°C overnight. Tissue sections were treated with their respective biotinylated secondary antibodies for 45 min at room temperature (Vector Laboratories; PK-6101 and BA-9400). Color was developed using the Vectastain ABC kit (Vector Laboratories) followed by diaminobenzidine (DAB) reaction. Sections were then counterstained with hematoxylin and dehydrated in ethanol and xylene. Images were acquired at $\times 20$ magnification using an Olympus microscope equipped with a D-26 color camera.

Immunofluorescence. Five-micrometer paraffin-embedded tissues were deparaffinized, epitope retrieved, and blocked in the same way as that for Ki-67 staining. The sections were covered with 1:50-diluted rabbit anti-human CD98 antibody (Santa Cruz Biotechnology; sc-9160), goat anti-mouse $\beta 1$ -integrin antibody (Santa Cruz Biotechnology; sc-6622), or rabbit anti-mouse mucin-2 antibody (Santa Cruz Biotechnology; sc-15334) in PBS and incubated overnight at 4°C. Tissues were washed with PBS and treated with anti-rabbit secondary antibody labeled with Alexa Fluor 488- and Alexa Fluor 568-phalloidin (Life Technologies) or with anti-rabbit secondary antibody labeled with Alexa Fluor 488 and anti-goat secondary antibody labeled with Alexa Fluor 568 (Life Technologies) in PBS for 1 h at room temperature. Sections were covered with mounting medium containing 4',6-diamidino-2-phenylindole (DAPI) (Vector Laboratories; H-1500) and coverslipped. Sections were viewed under an Olympus fluorescence microscope, and images were acquired at $\times 20$ magnification with a Hamamatsu digital camera (Orca-03G).

MPO assay. Colonic tissues were homogenized in ice-cold potassium phosphate buffer (50 mM K_2HPO_4 and 50 mM KH_2PO_4 [pH 6.0]) containing 0.5% hexadecyltrimethylammonium bromide (Sigma, St. Louis, MO). The homogenates were sonicated, freeze-thawed three times, and centrifuged at maximum speed for 15 min. Supernatants (20 μ l) or myeloperoxidase (MPO) standard was added to 1 mg/ml *o*-dianisidine hydrochloride (Sigma) and 0.0005% hydrogen peroxide. Absorbance was measured at 460 nm using a Synergy 2 microplate reader (BioTek). One unit of MPO activity was defined as the amount that degraded 1 μ mol of peroxidase per minute. The results were expressed as absorbance per milligram of protein.

Preparation of gold chips used to detect SPR. For surface plasmon resonance (SPR) experiments, we used gold film applied as a coating onto BK7 glass slides (Biosensing Instruments, Tempe, AZ). Each gold chip was cleaned with pure ethanol, dried under a stream of nitrogen (N_2), and treated with cystamine dihydrochloride (20 mM; Sigma) as described previously (37). After a chip was placed into the BI-2000 SPR machine, each gold biosensor chip covered with carboxy dextran was activated using a mixture of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide-*N*-hydroxysuccinimide (EDC-NHS) to form amide linkages between purified protein and the chip. Three successive injections of 10 μ g/ml full-length recombinant hCD98 or 10 μ g/ml recombinant hCD98 extracellular domain were performed to ensure sufficient protein adsorption to the chip. The coating of the chip with the protein is adequate to perform the experiment when the deviation of the resonance angle reaches 80 to 120 millidegrees (mDeg). As a protein control, one single injection of 100 μ g/ml of BSA on a separate channel of the same chip was enough to reach 80 to 120 mDeg. After adsorption of the two recombinant proteins or BSA, 1 M ethanolamine was injected to block unreacted carboxy dextran. Covalent linkage of recombinant proteins can be quantified by measuring the laser deflection value (Δ), which is directly proportional to the number of bound molecules, regardless of molecular weight or molecular length. Two fluidic channels were used on this instrument. The same sample plug flowed from the first to the second channel. When "serial mode" was selected, the BI-2000 machine injected both channels simultaneously with the same solution of bacteria. This option was essential for an accurate comparison of the interaction between full-length hCD98 (channel 1) or extracellular hCD98 (channel 2) and the designated bacteria.

Binding affinity. SPR is based on excitation and detection of the collective oscillations of free electrons in a metal film. Such electron oscillations are termed surface plasmons. A light is focused on the film through a glass prism, and reflected light is detected. At the resonance angle, plasmons absorb light and a dark line is seen in the reflected beam. Any molecular binding event taking place on or near the metal film causes a shift in the resonance angle. By assessing shift over time, molecular binding events can be monitored and the kinetics of binding events can be analyzed, without the use of labels.

Angular shift in degrees is the shift in resonance angle upon molecular adsorption or change in the refractive index of the solution. The refractive index unit (RIU) reflects a change in solution refractive index or development of any other process inducing an alteration in SPR signal equivalent to a variation in RIU. The resonance unit (RU) reflects adsorption at a

FIG 1 Expression of hCD98 and β 1-integrin in the colonic apical membranes of hCD98 Tg mice. (A) Western blotting of total colonic lysates from WT and hCD98 Tg mice. Total colonic protein (25 μ g/lane) was resolved by SDS-PAGE on a 4 to 15% gradient gel under reducing conditions followed by transfer to a nitrocellulose membrane. The blot was immunostained with a rabbit polyclonal anti-CD98 antibody. As a loading control, the same blot was stripped and reprobed with an antibody against β -actin. (B) Immunofluorescence staining of colonic tissue from WT and hCD98 Tg mice with an anti-hCD98 antibody showed apical localization of hCD98 (green) in the colon of hCD98 Tg mice. The cytoskeleton was visualized by staining for F-actin with Alexa Fluor 568-phalloidin (red), and the nucleus was stained with 4',6-diamidino-2-phenylindole (DAPI; blue). (C to F) Immunofluorescence staining of colonic tissue from WT and hCD98 Tg mice with an anti- β 1-integrin antibody showed migration of β 1-integrin (red) expression from crypt in WT mice to villus in Tg mice where hCD98 (green) is expressed. Colocalization of hCD98 and β 1-integrin can be seen in panel F (yellow and arrows). Original magnification, $\times 20$.

chip surface that causes a change in an SPR signal, and the unit is the “resonance unit” (RU). One RU is equivalent to 1 picogram per square millimeter of sensor surface. The three parameters may be interconverted as follows: 10^{-6} RIU = 0.73 RU = 7.3×10^{-5} degree = 0.73 pg/mm²; 0.1 mDeg = 1 pg/mm². Conversion from mDeg or RU to RIU assumes use of

a buffer with an index of refraction close to 1.33, employment of a BK7 prism, and use of a gold-coated sensor chip.

Briefly, after the chip was coated with full-length CD98, extracellular CD98 (Novus Biologicals, Littleton, CO; H00006520-P01/Q01), or BSA, bacterial solutions of increasing concentration were passed over the chip.

FIG 2 Intestinal epithelial CD98 overexpression increases *C. rodentium* adherence to mouse colon. *In vivo* colonization by *C. rodentium* in the colons of WT and hCD98 Tg mice was quantified by measuring CFU per gram of colon tissue. Significantly more colonization by *C. rodentium* was observed in the colons of hCD98 Tg mice than in those of WT mice (**, $P < 0.005$; $n = 9/\text{group}$).

A two-step interaction curve was obtained. The first step involved adsorption of bacteria to the maximal level. In the second step, when the flow of bacterial concentration returned to zero, nonspecific adsorbed bacteria were released with the running buffer. On the chip, only bacteria linked with CD98 or extracellular CD98 remained “attached.” The deviation of the resonance angle thus decreased to a plateau located at a level above the initial baseline. As our calculation model is based on a “one ligand, one receptor” method, we showed only the laser deflection directly correlated to the binding constant. We know that one bacterium may interact with more than one receptor and that stoichiometry of interaction is not known. Thus, we decided to use the laser deviation as the optimal parameter for the binding affinity. All comparisons between the different bacteria and recombinant proteins were performed as a measure of the laser deviation (mDeg).

Statistical analysis. Values were expressed as means $\pm$ standard errors of the means (SEM). Statistical analysis was performed using an unpaired two-tailed *t* test with GraphPad Prism 5 software. *P* values of <0.05 were considered statistically significant.

RESULTS

hCD98 is expressed apically in colonic epithelial cells of hCD98 Tg mice. Recently, we produced hCD98 Tg mice that express

FIG 3 Intestinal epithelial CD98 overexpression aggravates *C. rodentium*-induced intestinal inflammation. (A) Neutrophil infiltration into the colons of WT and hCD98 Tg mice, quantified by measuring MPO activity (units per milligram protein). Values represent means $\pm$ SEM (*, $P < 0.05$; $n = 6/\text{group}$). (B) Immunostaining of colonic tissue sections from WT and Tg control and infected mice with anti-Ly6g, a neutrophil marker. Bar, 50 μm ; original magnification, $\times 20$. (C) Representative H&E staining of colonic sections of WT and hCD98 Tg mice infected with *C. rodentium* and control (uninfected) mice. Bar, 50 μm ; original magnification, $\times 20$. (D to J) mRNA expression levels of proinflammatory (D to H) and anti-inflammatory (I and J) cytokines determined by real-time RT-PCR in WT and hCD98 Tg mice with or without *C. rodentium* infection. Values represent means $\pm$ SEM (*, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$; ns, nonsignificant; $n = 6/\text{group}$).

FIG 4 Intestinal epithelial CD98 overexpression induces proliferation in mouse colonic epithelial cells during *C. rodentium* infection. (A) Immunostaining of colonic tissue sections from WT and hCD98 Tg mice with anti-Ki-67 antibody. Bar, 50 μ m; original magnification, $\times 20$. (B) Ki-67-positive cells/crypt in WT and hCD98 Tg mice. Greater numbers of Ki-67-positive cells were detected in colon sections from *C. rodentium*-infected hCD98 Tg mice than in those from WT mice. (C and D) mRNA and protein expression levels of cyclin D1 determined by RT-PCR and Western blotting, respectively. Values represent means $\pm$ SEM (*, $P < 0.05$; ***, $P < 0.001$; $n = 6$ /group).

hCD98 under the control of the villin promoter, resulting in specific overexpression of hCD98 in intestinal epithelia, including colonic epithelial cells. As shown in Fig. 1A, an antibody specific for hCD98 detected a CD98-reactive band in transgenic mouse colon tissue but not in tissue from WT mice, indicating that this antibody is not cross-reactive with mouse CD98. The observed molecular mass of hCD98 under reducing conditions (i.e., in the presence of β -mercaptoethanol) in intestinal epithelial cells of hCD98 Tg mice was ~ 85 kDa, which corresponds with the known molecular mass of monomeric glycosylated CD98. This antibody was used for subsequent immunofluorescence studies in WT and hCD98 Tg colon tissue. We and others have demonstrated that CD98 is normally expressed in the basolateral aspect of intestinal epithelial cells (22, 33, 35). However, as seen in Fig. 1B, hCD98 was aberrantly expressed in the apical plasma membrane of epithelial cells toward the luminal side in the colon of hCD98 Tg mice. This indicates that hCD98 is overexpressed on colonic epithelial cells near the lumen in transgenic mice, giving it direct access and an opportunity to interact with bacteria and other digested food products passing through the colon.

Intestinal epithelial CD98 overexpression induces $\beta 1$ -integrin expression in well-differentiated surface cells. We have previously shown that intestinal epithelial CD98 has higher expression levels in undifferentiated crypt cells than in well-differentiated surface cells in wild-type mice (22). $\beta 1$ -Integrin presents the same expression pattern as does CD98, as shown in Fig. 1C. In contrast, we demonstrate that the expression of hCD98 and $\beta 1$ -integrin was higher in differentiated cells (surface cells) than in undifferentiated cells (crypt cells) in hCD98 Tg mice (Fig. 1D and E). In addition, we also observed that $\beta 1$ -integrin and CD98 were colocalized in the apical plasma membrane in well-differentiated cells (surface cells) (Fig. 1F, see arrow). This shows that CD98 overexpression induces $\beta 1$ -integrin expression in well-differentiated cells while reducing its expression in undifferentiated cells. We do not yet understand the mechanism through which CD98 overexpression changes the expression gradient of $\beta 1$ -integrin along the crypt-surface axis. However, we demonstrate that CD98 and $\beta 1$ -integrin are colocalized in well-differentiated cells, suggesting the close relationship between hCD98 overexpression and $\beta 1$ -integrin activity.

FIG 5 Intestinal epithelial CD98 overexpression increases EPEC adherence and EPEC-induced proinflammatory cytokine production and decreases anti-inflammatory cytokine responses in cultured mouse colons. (A) *Ex vivo* colonization of EPEC in colons of WT and hCD98 Tg mice, quantified as CFU per gram of colon tissue. Significantly more colonization of EPEC was observed in hCD98 Tg mouse colons than in WT mouse colons (*, $P < 0.05$; $n = 4$ /group). Experiments were performed in duplicate. (B to G) mRNA expression levels of proinflammatory (B to E) and anti-inflammatory (F and G) cytokines determined by real-time RT-PCR. Values represent means $\pm$ SEM (*, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.001$; $n = 6$ /group). (H) Cyclin D1 protein expression levels in EPEC-infected cultured colons of WT and hCD98 Tg mice as determined by Western blotting. Total colonic protein from WT and hCD98 Tg mice with or without EPEC infection (50 μ g/lane) was resolved by SDS-PAGE on a 7.5% gel followed by transfer to a nitrocellulose membrane. The blot was immunostained with a rabbit polyclonal anti-cyclin D1 antibody. As a loading control, the same blot was stripped and reprobbed with an antibody against β -actin.

Intestinal epithelial CD98 overexpression increases colonization of the colon by *C. rodentium*. The role of CD98 in host responses to *C. rodentium* infection was assessed by infecting hCD98 Tg mice with *C. rodentium*. Figure 2 shows that *C. rodentium* colonization of hCD98 Tg mouse colons was increased compared with colonization in those of WT mice (3×10^{10} versus 8.7×10^8 average CFU/g of colonic tissue in hCD98 Tg versus WT mice, respectively). We also demonstrated that the location and amount of mucin-2 (marker of mucosa) are not altered in hCD98 Tg mouse colon tissue compared to WT tissue (see Fig. S1 in the supplemental material), suggesting that the increased colonization of *C. rodentium* in Tg mice does not come from a modification of the mucosa. This indicates that intestinal epithelial CD98 might be involved in increasing the adherence of *C. rodentium* to colonic cells.

Intestinal epithelial CD98 overexpression increases *C. rodentium*-induced intestinal inflammation. CD98 overexpression resulted in increased colonization of *C. rodentium* in the mouse colon, and previous studies have shown that *C. rodentium* infection and colonization of intestinal epithelial cells result in epithelial hyperplasia and mucosal inflammation in mice (38, 39). To elucidate the effect of hCD98 overexpression on *C. rodentium*-induced inflammation, we assessed the expression of various pro- and anti-inflammatory markers in WT and hCD98 Tg mice infected with *C. rodentium*. Measurements of colonic myeloperoxidase (MPO) activity showed increased neutrophil accumulation in hCD98 Tg mouse colons compared with that in WT mice (Fig. 3A). We further show a higher infiltration of neutrophils in

hCD98 Tg mice than in WT mice, using the neutrophil marker Ly6g (Fig. 3B). This is consistent with the MPO levels previously observed. Examination of H&E-stained colonic tissues from control groups revealed no morphological differences between WT

FIG 6 Intestinal epithelial CD98 knockdown decreases EPEC adherence in Caco2-BBE intestinal cell lines. *In vitro* adherence of EPEC in Caco2-BBE cell lines treated with CD98 siRNA, no-siRNA control, and negative siRNA control was quantified by measuring CFU per ml of cell lysate. EPEC adherence decreased in Caco2-BBE cells treated with CD98 siRNA compared to nontreated cells or cells treated with negative siRNA control (**, $P < 0.005$; $n = 5$ or 3 /group).

FIG 7 Schematic representation and Western blotting of recombinant hCD98 full-length and extracellular proteins. (A) GST-tagged hCD98 full-length protein and 110 amino acids (from 203 to 313) of the extracellular domain were used in Western blot and SPR experiments. (B) Western blotting of GST-tagged full-length hCD98 and extracellular protein (50 ng/lane) was resolved by SDS-PAGE on a 4 to 15% gradient gel under reducing conditions followed by transfer to a nitrocellulose membrane. The blot was immunostained with an anti-human GST tag antibody. An immunoreactive band around 75 kDa was observed for GST-tagged full-length hCD98 protein, while a 37-kDa band was observed for GST-tagged extracellular hCD98 protein.

and hCD98 Tg mice (Fig. 3C). Seven days after *C. rodentium* infection, an increase in crypt height indicative of hyperplasia, a typical characteristic of *C. rodentium* infection (40), was evident in both WT and hCD98 Tg mice (Fig. 3C). Apart from the significant increase in neutrophil infiltration in hCD98 Tg mice compared with WT mice, which correlated with the increase in MPO activity and Ly6g staining, morphological differences between the colons of these mice were relatively modest.

In addition, compared with WT mice, hCD98 Tg mice exhibited increased colonic production of murine keratinocyte-derived chemokine (KC) mRNA in response to *C. rodentium* infection (Fig. 3D). Colonic levels of the proinflammatory cytokines interleukin-6 (IL-6), IL-17a, tumor necrosis factor alpha (TNF- α), and gamma interferon (IFN- γ) in hCD98 Tg mice were also increased compared with those observed in WT mice (Fig. 3E to H). Production of the anti-inflammatory cytokines IL-10 and transforming growth factor β (TGF- β) in the colons of hCD98 Tg mice was lower than that in WT mice (Fig. 3I and J), suggesting that endogenous anti-inflammatory mechanisms might be more effectively inactivated in hCD98 Tg mice. Collectively, our data suggest that colonic CD98 may have a role in colonic infections by modulating bacterial-epithelial interactions and inflammatory responses to pathogenic bacteria.

Intestinal epithelial CD98 overexpression increases cell proliferation in the colon during *C. rodentium* infection. As noted above, CD98 overexpression in IECs resulted in increased colonization and production of proinflammatory cytokines during *C. rodentium* infection. Because CD98 is known to enhance prosurvival signaling (28, 41), we examined the effect of *C. rodentium* infection on expression of the proliferative markers Ki-67 and cyclin D1 in WT and hCD98 Tg mice. As shown in Fig. 4A, *C. rodentium* infection resulted in increased numbers of Ki-67-positive cells in both WT and hCD98 Tg mice compared to control

(uninfected) mice. There was also a marked increase in Ki-67-positive cells in infected colon tissue of hCD98 Tg mice compared to WT infected tissue (Fig. 4A and B). Cyclin D1 mRNA levels were also increased in colon tissues of *C. rodentium*-infected WT and hCD98 Tg mice (Fig. 4C). Cyclin D1 protein levels were significantly higher in the colons of infected hCD98 Tg mice than in those of Tg control mice (Fig. 4D). Taken together, these results suggest that CD98-dependent cell proliferation may play a role in colonic cells during *C. rodentium* infection.

Intestinal epithelial CD98 overexpression increases EPEC adherence to colonic tissue. Since EPEC is in the same group of A/E pathogens as *C. rodentium*, we sought to examine the effect of intestinal epithelial hCD98 overexpression on EPEC adherence and EPEC-induced inflammation in colonic cells. Figure 5A shows an increase in EPEC adherence to cultured colonic tissues from hCD98 Tg mice compared with those from WT mice 6 h after infection. In addition, cultured colons from hCD98 Tg mice produced higher mRNA levels of KC and proinflammatory cytokines, such as TNF- α , IFN- γ , and IL-6, than did those from uninfected hCD98 Tg control mice (Fig. 5B to E). IL-6 levels were also significantly higher in EPEC-infected hCD98 Tg colonic tissue than in WT infected tissue. Levels of the anti-inflammatory cytokines IL-10 and TGF- β were lower in EPEC-infected hCD98 Tg colonic tissues than in WT tissues (Fig. 5F and G). In addition, cyclin D1 protein levels were higher in infected colon tissue of hCD98 Tg mice than in WT infected tissue (Fig. 5H). The results of these *ex vivo* experiments with EPEC infection correlated well with *in vivo* data, indicating that CD98 overexpression increases colonization of A/E bacteria and enhances the inflammatory response in colonic tissue.

CD98 knockdown decreases adherence of EPEC in intestinal epithelial cells. *In vivo* and *ex vivo* studies showed that CD98 overexpression increased the adherence of enteropathogenic bac-

FIG 8 Recombinant full-length hCD98 and hCD98 extracellular domain bind to *C. rodentium* and EPEC. (A to C) Sensorgrams show covalent linkage of recombinant full-length GST-hCD98 (A), extracellular GST-hCD98 (B), or BSA (C) onto carboxy dextran chips. Several injections of 10 μ g/ml recombinant full-length GST-hCD98, 10 μ g/ml extracellular GST-hCD98, or 100 μ g/ml BSA were performed prior to SPR measurement and further loadings. The first injection of bound recombinant full-length GST-hCD98 resulted in a laser deflection of $\Delta = 37.1$ mDeg. After the first injection, adsorbed extracellular GST-hCD98 caused a laser deflection of $\Delta' = 38.9$ mDeg. After one injection of BSA, the adsorption caused a laser deflection of $\Delta'' = 132.9$ mDeg. After adsorption of the recombinant full-length GST-CD98, extracellular GST-hCD98, or BSA, injection of 1 M ethanolamine was performed to remove excess carboxy dextran. (D and F) *C. rodentium* (2×10^{10} to 5×10^{10} CFU/ml) binding to recombinant full-length GST-hCD98 and GST-hCD98 extracellular domain resulted in a resonance angle deflection. (E and G) EPEC (2×10^{10} , 3×10^{10} , 4×10^{10} , and 6×10^{10} CFU/ml) binding to recombinant full-length GST-hCD98 or GST-hCD98 extracellular domain resulting in a resonance angle deflection. (H) Binding of recombinant full-length GST-hCD98, GST-hCD98 extracellular domain, or BSA to *C. rodentium*, EPEC, and EPEC Δeae mutant, measured in mDeg, as determined by SPR. Representative traces of four independent experiments are shown. Values represent means $\pm$ SEM (***, $P < 0.001$; $n = 3$ /group).

terea in colonic tissue. We additionally demonstrate a significant reduction of EPEC adherence in CD98 siRNA-transfected Caco2-BBE cells (a nongoblet cell line; 71.7% reduction in CD98 expression by siRNA) compared to untransfected Caco2-BBE cells and Caco2-BBE cells transfected with Stealth RNAi (negative control) (Fig. 6). Taken together, these results indicate that CD98 increases the adherence of enteropathogenic bacteria in intestinal epithelial cells and further suggest that intestinal epithelial CD98 directly contributes to colonization.

Recombinant hCD98 extracellular domain interacts directly with *C. rodentium* and EPEC. To date, direct binding and/or interaction between CD98 and *C. rodentium* or between CD98 and EPEC has not been quantitatively studied. To address these possible interactions, we used surface plasmon resonance (SPR) (42). The GST-tagged CD98 full-length and extracellular proteins (Fig. 7A) used in SPR studies were first tested with Western blotting using anti-GST antibody. A reactive band of GST-tagged hCD98 full-length protein showed up at around 75 kDa while GST-tagged hCD98 extracellular protein was observed at 37 kDa (Fig. 7B). As SPR studies showed (Fig. 8A), adsorption of full-length hCD98 created a laser deflection of 37.1 mDeg (Δ) for the first injection of the recombinant protein, corresponding to 371 pg/mm^2 of adsorbed protein ($0.1 \text{ mDeg} = 1 \text{ pg}/\text{mm}^2$). Several

injections were then performed to reach 80 to 120 mDeg of deviation of the resonance angle. Linkage of the recombinant hCD98 extracellular domain resulted in a laser deflection of 38.9 mDeg (Δ') for the first injection, equivalent to 389 pg/mm^2 of bound protein (Fig. 8B), whereas adsorption of BSA produced a laser deflection of 132.9 mDeg (Δ'') (Fig. 8C). Three successive injections of protein led to a total deflection of approximately 80 mDeg, corresponding to 800 pg/mm^2 for both recombinant proteins; a total deflection of 132.9 mDeg was achieved with one injection of BSA. Thus, the chip was optimally coated with full-length hCD98, hCD98 extracellular domain, or BSA, and no additional covalent bonds could be formed between an analyte and the chip.

To examine direct interactions between CD98 and *C. rodentium* or EPEC, we performed SPR assays using recombinant full-length hCD98 or hCD98 extracellular domain as the bound ligand and *C. rodentium*, EPEC, or an apathogenic EPEC Δeae mutant (negative control) at three different concentrations (2×10^{10} , 3×10^{10} , and 6×10^{10} CFU/ml) as analytes. First we observed that *C. rodentium* and EPEC bound directly to immobilized full-length CD98 recombinant protein, and the deflection of the resonance angle was related to *C. rodentium* or EPEC concentration (Fig. 8D and E). Thus, for 3×10^{10} CFU/ml, the deflection was 72.2 mDeg

FIG 9 Model showing the effect of CD98 overexpression on enteropathogenic bacterial infection and inflammation in WT and Tg hCD98 mouse IECs. The Tg mouse model mimics the condition observed in diseases such as inflammatory bowel disease (IBD) where CD98 expression is upregulated. In Tg mice, there is loss of barrier function and polarity, resulting in redistribution of basolateral proteins such as CD98 and β 1-integrin. Exposure of CD98 toward the lumen gives the enteropathogenic bacteria direct access to CD98, resulting in direct interaction between the protein and bacteria, which in turn results in more attachment and bacterially induced inflammation in Tg mice than in WT mice.

for *C. rodentium* and 86.1 mDeg for EPEC (Fig. 8H). We repeated the experiment using recombinant hCD98 extracellular domain as the bound ligand. Interestingly, binding of the hCD98 extracellular domain to *C. rodentium* (73.6 mDeg) and EPEC (126.8 mDeg) was similar to that of full-length hCD98 (Fig. 8F, G, and H). Minimal or no binding of *C. rodentium* or EPEC to BSA was observed compared to full-length hCD98 or extracellular domain (Fig. 8H); also, the EPEC Δeae mutant had minimal binding values of 1.9, 2.7, and 4.15 to hCD98 full-length protein, extracellular domain, and BSA, respectively. Taken together, these results show that both recombinant full-length hCD98 and hCD98 extracellular domain bind similarly and directly to *C. rodentium* and EPEC.

DISCUSSION

In a normal physiological context, CD98 is expressed in the basolateral domain of colonocytes (22). However, in hCD98 Tg mice, immunofluorescence revealed that hCD98 was expressed at the apical region of colonic epithelial cells. In a previous study, we demonstrated that IEC-specific CD98 overexpression induced barrier dysfunction and perturbed inflammatory responses, both of which are known to contribute to the pathophysiology of irritable bowel disease (35). Furthermore, during intestinal inflammation, subcellular localization of membrane-bound proteins is deregulated in polarized intestinal cells, resulting in apical plasma

expression of membrane-bound molecules such as β 1-integrin that are normally segregated to the basolateral domain. In the latter context, it is not surprising that CD98, which is a β 1-integrin partner, follows β 1-integrin deregulated expression during intestinal inflammation. The apical localization of CD98 suggests that CD98 could interact with bacteria that are present in the intestinal lumen.

Infection with *C. rodentium* resulted in increased bacterial colonization of hCD98 Tg mice, compared to WT mice. hCD98 Tg mice also exhibited an increase in *C. rodentium*-induced production of KC and other proinflammatory cytokines, such as IL-6, IL-17a, TNF- α , and IFN- γ , in the colon. A previous study has shown the importance of IL-6 in host defense responses to A/E pathogenic infections in mouse colonic tissue (43); also interestingly, IL-17 was shown to be a key cytokine in inflammation and host defense responses to bacterial infection (44). These results are in agreement with our MPO assays and Ly6g staining, which showed greater neutrophil infiltration in infected colonic tissue of hCD98 Tg than in that of WT mice. This suggests that endogenous proinflammatory molecules are more effectively activated in hCD98 Tg mice upon *C. rodentium* infection. The increase in proinflammatory cytokine expression was associated with decreased colonic anti-inflammatory cytokine production levels in

hCD98 Tg mice compared to those in WT mice. Taken together, these results demonstrate that intestinal epithelial CD98 participates in host resistance mechanisms through the production of cytokines that recruit phagocytic cells to the site of infection to combat the pathogen.

Cell proliferation markers were significantly upregulated in *C. rodentium*-infected hCD98 Tg mice compared to WT mice, demonstrating the involvement of CD98 in cell proliferation during *C. rodentium* infection. *Ex vivo* studies using colonic tissues of hCD98 Tg and WT mice infected with the human pathogen EPEC (the CD98 protein overexpressed in transgenic mice colon is of human origin) clearly supported the *in vivo* data obtained from *C. rodentium* infection studies. Together, these results suggest that intestinal epithelial CD98 is involved in the rapid turnover and exfoliation of mucosal epithelial cells, providing an innate tolerance defense system against bacterial infection.

Intestinal epithelial CD98, a transmembrane protein, interacts directly with β 1-integrin, and this interaction is important for integrin signaling, which controls a number of biological processes, such as cell proliferation, survival, and epithelial cell adhesion/polarity (26, 33, 35, 45). In addition, we have previously demonstrated that hCD98 coimmunoprecipitates with β 1-integrin in colonic epithelial cells (33), reflecting colonization of CD98 and β 1-integrin in apical colonic membranes. On the basis of our observation that EPEC and *C. rodentium* can interact with CD98 and/or β 1-integrin, we suggest that pathogen-CD98 interactions could regulate β 1-integrin activation.

In vitro SPR experiments showed that full-length recombinant hCD98 was able to bind to both *C. rodentium* and EPEC. Remarkably, the first 103 amino acid residues of the extracellular CD98 loop were sufficient for binding to EPEC and *C. rodentium*. The involvement of the CD98 extracellular loop in CD98 function has been previously demonstrated. For example, it has been shown that ectophosphorylation of CD98 regulates cell-cell interactions (46), the extracellular CD98 loop is required for early murine development (47), and extracellular CD98 is involved in PDZ domain-dependent interactions with extracellular proteins (48). Interestingly, our study supports a recent report showing that EspZ, one of the effectors of EPEC, binds to CD98 in host cells during infection in an *in vitro* model (34).

We have recently demonstrated that intestinal epithelial CD98 expression regulates the intestinal barrier function (35). Our laboratory and other researchers have also shown that CD98 expression is upregulated during intestinal inflammation (21–24). As summarized in our proposed model (Fig. 9), we suggest that there is a regulatory link between barrier function and the inflammation-mediated upregulation of intestinal CD98, leading to the redistribution of basolateral membrane proteins. This redistribution of proteins such as CD98/ β 1-integrin to the apical side of intestinal epithelial cells could directly modulate innate host responses to enteric bacterial pathogens.

ACKNOWLEDGMENTS

This work was supported by grants from the Department of Veterans Affairs and by the National Institute of Diabetes and Digestive and Kidney Diseases (National Institutes of Health) through grant RO1-DK-071594 (to D.M.) and grant K01-DK097192 (to H.L.).

REFERENCES

- Danielsen EM, Hansen GH. 2008. Lipid raft organization and function in the small intestinal brush border. *J. Physiol. Biochem.* 64:377–382.
- Izumi Y, Hirose T, Tamai Y, Hirai S, Nagashima Y, Fujimoto T, Tabuse Y, Kempfues KJ, Ohno S. 1998. An atypical PKC directly associates and colocalizes at the epithelial tight junction with ASIP, a mammalian homologue of *Caenorhabditis elegans* polarity protein PAR-3. *J. Cell Biol.* 143:95–106.
- Baas AF, Kuipers J, van der Wel NN, Batlle E, Koerten HK, Peters PJ, Clevers HC. 2004. Complete polarization of single intestinal epithelial cells upon activation of LKB1 by STRAD. *Cell* 116:457–466.
- Man Y, Hart VJ, Ring CJ, Sanjar S, West MR. 2000. Loss of epithelial integrity resulting from E-cadherin dysfunction predisposes airway epithelial cells to adenoviral infection. *Am. J. Respir. Cell Mol. Biol.* 23:610–617.
- Fujimori T, Takeichi M. 1993. Disruption of epithelial cell-cell adhesion by exogenous expression of a mutated nonfunctional N-cadherin. *Mol. Biol. Cell* 4:37–47.
- Troxell ML, Gopalakrishnan S, McCormack J, Poteat BA, Pennington J, Garringer SM, Schneeberger EE, Nelson WJ, Marrs JA. 2000. Inhibiting cadherin function by dominant mutant E-cadherin expression increases the extent of tight junction assembly. *J. Cell Sci.* 113:985–996.
- Nava P, Lopez S, Arias CF, Islas S, Gonzalez-Mariscal L. 2004. The rotavirus surface protein VP8 modulates the gate and fence function of tight junctions in epithelial cells. *J. Cell Sci.* 117:5509–5519.
- Beau I, Cotte-Laffitte J, Amsellem R, Servin AL. 2007. A protein kinase A-dependent mechanism by which rotavirus affects the distribution and mRNA level of the functional tight junction-associated protein, occludin, in human differentiated intestinal Caco-2 cells. *J. Virol.* 81:8579–8586.
- Evans MJ, von Hahn T, Tscherne DM, Syder AJ, Panis M, Wolk B, Hatzioannou T, McKeating JA, Bieniasz PD, Rice CM. 2007. Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. *Nature* 446:801–805.
- Kerr JR. 1999. Cell adhesion molecules in the pathogenesis of and host defence against microbial infection. *Mol. Pathol.* 52:220–230.
- Knodler LA, Celli J, Finlay BB. 2001. Pathogenic trickery: deception of host cell processes. *Nat. Rev. Mol. Cell Biol.* 2:578–588.
- Chen HD, Frankel G. 2005. Enteropathogenic *Escherichia coli*: unravelling pathogenesis. *FEMS Microbiol. Rev.* 29:83–98.
- Miyake M, Hanajima M, Matsuzawa T, Kobayashi C, Minami M, Abe A, Horiguchi Y. 2005. Binding of intimin with Tir on the bacterial surface is prerequisite for the barrier disruption induced by enteropathogenic *Escherichia coli*. *Biochem. Biophys. Res. Commun.* 337:922–927.
- Guttman JA, Finlay BB. 2009. Tight junctions as targets of infectious agents. *Biochim. Biophys. Acta* 1788:832–841.
- Knutton S, Rosenshine I, Pallen MJ, Nisan I, Neves BC, Bain C, Wolff C, Dougan G, Frankel G. 1998. A novel EspA-associated surface organelle of enteropathogenic *Escherichia coli* involved in protein translocation into epithelial cells. *EMBO J.* 17:2166–2176.
- Kenny B, DeVinney R, Stein M, Reinscheid DJ, Frey EA, Finlay BB. 1997. Enteropathogenic *E. coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells. *Cell* 91:511–520.
- Sekiya K, Ohishi M, Ogino T, Tamano K, Sasakawa C, Abe A. 2001. Supermolecular structure of the enteropathogenic *Escherichia coli* type III secretion system and its direct interaction with the EspA-sheath-like structure. *Proc. Natl. Acad. Sci. U. S. A.* 98:11638–11643.
- Wachter C, Beinke C, Mattes M, Schmidt MA. 1999. Insertion of EspD into epithelial target cell membranes by infecting enteropathogenic *Escherichia coli*. *Mol. Microbiol.* 31:1695–1707.
- Frankel G, Lider O, Hershkovich R, Mould AP, Kachalsky SG, Candy DC, Cahalon L, Humphries MJ, Dougan G. 1996. The cell-binding domain of intimin from enteropathogenic *Escherichia coli* binds to beta1 integrins. *J. Biol. Chem.* 271:20359–20364.
- Muza-Moons MM, Koutsouris A, Hecht G. 2003. Disruption of cell polarity by enteropathogenic *Escherichia coli* enables basolateral membrane proteins to migrate apically and to potentiate physiological consequences. *Infect. Immun.* 71:7069–7078.
- Kucharzik T, Luger A, Yan Y, Driss A, Charrier L, Sitaraman S, Merlin D. 2005. Activation of epithelial CD98 glycoprotein perpetuates colonic inflammation. *Lab. Invest.* 85:932–941.
- Nguyen HT, Dalmasso G, Yan Y, Laroui H, Dahan S, Mayer L, Sitaraman SV, Merlin D. 2010. MicroRNA-7 modulates CD98 expression during intestinal epithelial cell differentiation. *J. Biol. Chem.* 285:1479–1489.
- Yan Y, Dalmasso G, Sitaraman S, Merlin D. 2007. Characterization of the human intestinal CD98 promoter and its regulation by interferon-gamma. *Am. J. Physiol. Gastrointest. Liver Physiol.* 292:G535–545.

24. Charania MA, Ayyadurai S, Ingersoll SA, Xiao B, Viennois E, Yan Y, Laroui H, Sitaraman SV, Merlin D. 2012. Intestinal epithelial CD98 synthesis specifically modulates expression of colonic microRNAs during colitis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 302:G1282–G1291.
25. Chandrasekaran S, Guo NH, Rodrigues RG, Kaiser J, Roberts DD. 1999. Pro-adhesive and chemotactic activities of thrombospondin-1 for breast carcinoma cells are mediated by alpha3beta1 integrin and regulated by insulin-like growth factor-1 and CD98. *J. Biol. Chem.* 274:11408–11416.
26. Fenczik CA, Sethi T, Ramos JW, Hughes PE, Ginsberg MH. 1997. Complementation of dominant suppression implicates CD98 in integrin activation. *Nature* 390:81–85.
27. Fenczik CA, Zent R, Dellos M, Calderwood DA, Satriano J, Kelly C, Ginsberg MH. 2001. Distinct domains of CD98hc regulate integrins and amino acid transport. *J. Biol. Chem.* 276:8746–8752.
28. Feral CC, Nishiya N, Fenczik CA, Stuhlmann H, Slepak M, Ginsberg MH. 2005. CD98hc (SLC3A2) mediates integrin signaling. *Proc. Natl. Acad. Sci. U. S. A.* 102:355–360.
29. Henderson NC, Collis EA, Mackinnon AC, Simpson KJ, Haslett C, Zent R, Ginsberg M, Sethi T. 2004. CD98hc (SLC3A2) interaction with beta 1 integrins is required for transformation. *J. Biol. Chem.* 279:54731–54741.
30. Kolesnikova TV, Mannion BA, Berditchevski F, Hemler ME. 2001. Beta1 integrins show specific association with CD98 protein in low density membranes. *BMC Biochem.* 2:10. doi:10.1186/1471-2091-2-10.
31. Liu S, Calderwood DA, Ginsberg MH. 2000. Integrin cytoplasmic domain-binding proteins. *J. Cell Sci.* 113:3563–3571.
32. Zent R, Fenczik CA, Calderwood DA, Liu S, Dellos M, Ginsberg MH. 2000. Class- and splice variant-specific association of CD98 with integrin beta cytoplasmic domains. *J. Biol. Chem.* 275:5059–5064.
33. Merlin D, Sitaraman S, Liu X, Eastburn K, Sun J, Kucharzik T, Lewis B, Madara JL. 2001. CD98-mediated links between amino acid transport and beta 1 integrin distribution in polarized columnar epithelia. *J. Biol. Chem.* 276:39282–39289.
34. Shames SR, Deng W, Guttman JA, de Hoog CL, Li Y, Hardwidge PR, Sham HP, Vallance BA, Foster LJ, Finlay BB. 2010. The pathogenic *E. coli* type III effector EspZ interacts with host CD98 and facilitates host cell prosurvival signalling. *Cell. Microbiol.* 12:1322–1339.
35. Nguyen HT, Dalmaso G, Torkvist L, Halfvarson J, Yan Y, Laroui H, Shmerling D, Tallone T, D'Amato M, Sitaraman SV, Merlin D. 2011. CD98 expression modulates intestinal homeostasis, inflammation, and colitis-associated cancer in mice. *J. Clin. Invest.* 121:1733–1747.
36. Nguyen HT, Dalmaso G, Powell KR, Yan Y, Bhatt S, Kalman D, Sitaraman SV, Merlin D. 2009. Pathogenic bacteria induce colonic PepT1 expression: an implication in host defense response. *Gastroenterology* 137:1435–1447.
37. Yao X, Li X, Toledo F, Zurita-Lopez C, Gutova M, Momand J, Zhou F. 2006. Sub-attomole oligonucleotide and p53 cDNA determinations via a high-resolution surface plasmon resonance combined with oligonucleotide-capped gold nanoparticle signal amplification. *Anal. Biochem.* 354:220–228.
38. Mundy R, MacDonald TT, Dougan G, Frankel G, Wiles S. 2005. *Citrobacter rodentium* of mice and man. *Cell. Microbiol.* 7:1697–1706.
39. Rodrigues DM, Sousa AJ, Johnson-Henry KC, Sherman PM, Gareau MG. 2012. Probiotics are effective for the prevention and treatment of *Citrobacter rodentium*-induced colitis in mice. *J. Infect. Dis.* 206:99–109.
40. Luperchio SA, Schauer DB. 2001. Molecular pathogenesis of *Citrobacter rodentium* and transmissible murine colonic hyperplasia. *Microbes Infect.* 3:333–340.
41. Cai S, Bulus N, Fonseca-Siesser PM, Chen D, Hanks SK, Pozzi A, Zent R. 2005. CD98 modulates integrin beta1 function in polarized epithelial cells. *J. Cell Sci.* 118:889–899.
42. Torrerri P, Ceccarini M, Macioce P, Petrucci TC. 2005. Biomolecular interactions by surface plasmon resonance technology. *Ann. Ist. Super. Sanita* 41:437–441.
43. Dann SM, Spehlmann ME, Hammond DC, Iimura M, Hase K, Choi LJ, Hanson E, Eckmann L. 2008. IL-6-dependent mucosal protection prevents establishment of a microbial niche for attaching/effacing lesion-forming enteric bacterial pathogens. *J. Immunol.* 180:6816–6826.
44. Geddes K, Rubino SJ, Magalhaes JG, Streutker C, Le Bourhis L, Cho JH, Robertson SJ, Kim CJ, Kaul R, Philpott DJ, Girardin SE. 2011. Identification of an innate T helper type 17 response to intestinal bacterial pathogens. *Nat. Med.* 17:837–844.
45. Cantor JM, Ginsberg MH, Rose DM. 2008. Integrin-associated proteins as potential therapeutic targets. *Immunol. Rev.* 223:236–251.
46. Nguyen HT, Dalmaso G, Yan Y, Obertone TS, Sitaraman SV, Merlin D. 2008. Ecto-phosphorylation of CD98 regulates cell-cell interactions. *PLoS One* 3:e3895. doi:10.1371/journal.pone.0003895.
47. Sato Y, Heimeier RA, Li C, Deng C, Shi YB. 2011. Extracellular domain of CD98hc is required for early murine development. *Cell Biosci.* 1:7. doi:10.1186/2045-3701-1-7.
48. Yan Y, Vasudevan S, Nguyen H, Bork U, Sitaraman S, Merlin D. 2007. Extracellular interaction between hCD98 and the PDZ class II domain of hCASK in intestinal epithelia. *J. Membr. Biol.* 215:15–26.