

Supporting Information

Barr et al. 10.1073/pnas.1305923110

SI Materials and Methods

Mucus Sample Collection. Mucus samples were collected directly from the surface of organisms using a syringe, swab, or custom suction device. Environmental samples were collected as close to the mucus sample as possible, typically within 30–50 cm of the mucosal surface. Specific organism details are as follows:

Sea anemones were sampled from tidal rock pools at Ocean Beach, San Diego, CA. Surface mucus was collected by a custom suction device that dislodges surface mucus using a stream of 0.02 μm filtered seawater; the environmental sample was seawater collected directly above the anemone.

Hard corals were sampled at the Birch Aquarium, San Diego, CA. Surface mucus was collected by syringe directly from coral surfaces; environmental water samples were collected directly above the coral.

The polychaete, along with surrounding water, was collected at Scripps Pier, San Diego, CA, and carefully transported to the laboratory in a container. Surface mucus was collected via syringe, and the environmental sample was seawater from the container.

Teleost surface mucus was sampled at the Birch Aquarium, San Diego, CA. Surface mucus was collected by custom suction device; the environmental water sample was collected directly above the teleost within its tank.

Human gum mucus was sampled from a male subject with no current pathology/disease. Surface mucus was collected by swab; the environmental sample was expectorated saliva. Consent was obtained for all human samples collected under the San Diego State University Institutional Review Board #2121.

Mouse intestine was excised from a healthy mouse. Surface mucus was collected by cutting open the intestine, washing the mucosal surface with 0.02 μm -filtered PBS buffer, then scraping off the mucus layer; the environmental sample was collected from the intestinal lumen directly adjacent to the sampled mucosa. All animal experiments were approved by the Committee on the Use and Care of Animals (SDSU, APF #10-08-024D) and performed using accepted veterinary standards.

Bacterial and Phage Counts from Mucus and Environmental Samples.

Samples of mucus and the adjacent environment were collected directly from nine evolutionarily diverse mucosal surfaces (Fig. S1). Samples were transported and maintained on ice until processed. All samples were fixed overnight in 0.5% glutaraldehyde at 4 $^{\circ}\text{C}$, then incubated in 6.5 mM DTT at 37 $^{\circ}\text{C}$ for 1 h to assist mucus degradation. A 1–100- μL aliquot was diluted with 2 mL of 0.02 μm SM buffer (100 mM NaCl, 8 mM MgSO_4 , 50 mM Tris-Cl, in dH_2O), briefly mixed, then filtered onto a 0.02- μm Anodisc polycarbonate filter (Whatman). Filters were stained with 10 \times SYBR Gold, washed, and visualized on a Zeiss epifluorescence microscope. For each sample, 20–30 images were taken for both bacteria and virus-like particles. Images were analyzed using Image-Pro Plus 5.1 software (MediaCybernetics). Counts of bacteria and virus-like particles (referred to as “phage” throughout the text) per milliliter were made as previously described (1).

Tissue Culture Cells and Mucus Reduction.

Monolayers of various mucus-producing and non-mucus-producing tissue culture (TC) cells were grown to confluence in six-well Multiwell tissue culture plates (Becton Dickinson). (i) Mucus-producing TC cells were exposed to 1 $\mu\text{g}/\text{mL}$ of a phorbol ester, phorbol-12-myristate 13-acetate (Sigma–Aldrich) in the culture media overnight to stimulate the mucin secretory response (2). (ii) The mucolytic agent *N*-acetyl-L-cysteine (NAC; Sigma–Aldrich) was used to

chemically remove mucus from A549 TC cells (60 mM NAC in serum-free media for 1 h with agitation) (3). Mucus depletion was confirmed using periodic acid-Schiff–Alcian blue (PAS/AB) (Fig. S2). (iii) A mucus-knockdown (*MUC*[−]) A549 cell line was produced by transduction of A549 cells with GIPZ Lentiviral Human MUC1 shRNA and TRIPZ Inducible Lentiviral Human MUC5AC shRNA as target vectors; an *shControl* A549 cell line was produced using the GIPZ Nonsilencing Lentiviral shRNA Control as a control vector (Thermo Scientific). Knockdown of mucus production in the *MUC*[−] cell line was confirmed by Western blot analysis and PAS/AB (Sigma–Aldrich; Figs. S3 and S4).

Transfection and Selection of A549 TC Mucus-Negative Clones.

A549 cells were transfected with GIPZ Lentiviral Human MUC1 shRNA and TRIPZ Inducible Lentiviral Human MUC5AC shRNA as target vectors or GIPZ Nonsilencing Lentiviral shRNA Control as a control vector (Thermo Scientific) according to the manufacturer’s instructions. Viral particles were produced by transfecting HEK 293T cells with a combination of plasmids containing 2 μg of packaging vector pCMV d8.2 containing the gag-pol proteins of HIV-1, 3 μg of the transfer vectors containing the LTRs of HIV-1, 3 μg of vesicular stomatitis virus envelope glycoprotein plasmid, and 1.5 μg of *pci*-HIV-1 viral protein R accessory protein plasmid. Growth medium was replaced 24 h post transfection, and viral supernatant was collected 48 and 72 h after transfection and then filtered through 0.45- μm polytetrafluoroethylene (PTFE) filters (Pall Corporation). A549 cells were seeded into six-well culture plates and grown to ~70% confluence. The cells then were washed twice in serum-free media before being incubated overnight in 1 mL of growth media and 1 mL of virus-containing media containing 5 $\mu\text{g}/\text{mL}$ of polybrene. The transduced cells subsequently were washed and cultured for 24 h in complete medium with 2 $\mu\text{g}/\text{mL}$ of doxycycline to induce expression of shRNA. Cells then were sorted using a BD FACSAria (BD Biosciences) at the San Diego State University Flow Cytometry Facility. A 100- μm nozzle was used at a sheath pressure of 20 psi. Excitation source was a 488-nm laser and emissions were collected using 530/30 band pass (BP) and 585/42 BP filters for GFP and red fluorescent protein, respectively. Between 10,000 and 300,000 cells were sorted for each population and collected in a 5-mL tube with 250 μL of FBS. The efficiency of MUC1-MUC5AC knockdown was confirmed by Western blot analysis and PAS/AB (Sigma–Aldrich), a stain for mucus-like substances.

Western Blot Analysis.

Expression of MUC1 (a membrane-tethered mucin) and MUC5AC (a secreted gel-forming mucin) was examined by Western blot analysis. *MUC*[−], *shControl*, and native A549 cell lines were grown to confluence and then lysed using radio-immunoprecipitation assay (RIPA) buffer (Thermo Scientific) containing 2 mM Na_3VO_4 , 100 mM NaF, 10 mM sodium pyrophosphate, 1 mM PMSF, and protease inhibitor mixture (Millipore). Aliquots containing 50 μg of total protein were subjected to SDS/PAGE, and the protein bands were transferred to a polyvinylidene difluoride membrane (Sigma–Aldrich). Membranes were blocked with 5% nonfat milk in Tris-buffered saline containing 0.05% Tween 20 at room temperature for 1 h and then incubated overnight at 4 $^{\circ}\text{C}$ with mouse anti-human MUC1 monoclonal antibody (clone S.854.6; Thermo Scientific), mouse anti-human MUC5AC monoclonal antibody (clone 2H7; Sigma–Aldrich), and rabbit anti-human GAPDH antibody (Millipore). After three washes, membranes were incubated for 1 h at room

temperature with anti-mouse or anti-rabbit IgG horseradish peroxidase-linked, species-specific, whole antibody (Fisher Scientific). Immunoreactivity was visualized and band intensity was normalized to the constitutively expressed GAPDH protein.

Multiple Particle Tracking. Assays were performed in plastic well chambers mounted on glass slides that had been coated with poly (dimethylsiloxane) to prevent phage adherence. Five microliters of 10^9 mL⁻¹ SYBR Gold-labeled phage suspensions was added to 50 μ L of 1% (wt/vol) mucin solution in 1 \times PBS buffer. Trajectories of fluorescently labeled phage were observed using a DeltaVision Spectris Model DV4 deconvolution microscope (Applied Precision) equipped with a 100 \times Olympus PlanApo 1.4 lens. Movies were captured using SoftWoRx 5.0.0 (Applied Precision): 100-ms temporal resolution for 30 s, 10 analyses per sample, $n > 100$ particle trajectories per analysis. Trajectories were analyzed with the ParticleTracker plugin for ImageJ (4). The coordinates of phage particle centroids were transformed into time-averaged mean square displacements: $\langle \Delta r^2(\tau) \rangle = \langle \Delta x^2 + \Delta y^2 \rangle$, from which effective diffusivities ($\langle D_{\text{eff}} \rangle$) were calculated; $D_{\text{eff}} = \langle \Delta r^2(\tau) \rangle / (4 \tau)$ (5, 6).

Glycan Microarray. Phage binding to glycans was assayed using printed mammalian glycan microarrays (version 5.1, Consortium for Functional Glycomics Core) containing 610 glycan targets. Samples of highly antigenic outer capsid protein (*hoc*⁺) T4 phage, *hoc*⁻ T4 phage, and buffer controls were applied to separate glycan microarray slides. Each slide received 35 μ L of sample, 35 μ L of binding buffer (Tris saline with 2 mM Ca²⁺, 2 mM Mg²⁺, 1% bovine serum albumin (BSA), and 0.05% Tween 20), and a coverslip. Slides first were incubated for 1 h at room temperature and washed with binding buffer. Slides then were incubated in SYBR Gold fluorescence dye (diluted 1:10,000 in binding buffer) for 1 h under a coverslip at room temperature, washed, dried, and immediately scanned in a PerkinElmer ProScanArray microarray scanner using an excitation wavelength of 488 nm. ImaGene software (BioDiscovery, Inc) was used to quantify fluorescence. Normalized relative fluorescence unit (RFU) values reported are the average (after subtraction of

background buffer fluorescence) from six spots for each glycan represented on the array.

Phylogenetic Analysis of Ig-Like Domains. The SEED database (www.theseed.org) collection of Ig-like polycystic kidney disease (PKD) protein families (Pfam) (PF00801) and the T4 Hoc sequence were searched against the 124 viral metagenomic datasets contained in the My Metagenome (MyMg) database (<http://edwards.sdsu.edu/cgi-bin/mymgdb/show.cgi>) using tBLASTn (PubMed accession numbers: 16336043, 17620602, 19156205, 19816605, 20547834, 17921274, 18441115, 19892985, 19555373, 20573248, 20631792, 21167942.79, 21193730.87, 21219518.96, 21245307.04, 21271095.12, 21296883.2, 21322671.29, 21348459.37, 21374247.45, 21400035.53; MG-Rast IDs: 21167942.79, 21193730.87, 21219518.96, 21245307.04, 21271095.12, 21296883.2, 21322671.29, 21348459.37, 21374247.45, 21400035.53).

Sequences with an e value of less than 1e-5 to Ig-like domains were retrieved. ORFs were called from the metagenome reads using Artemis (Wellcome Trust Sanger Institute); their position in the FASTA file is shown in Table S2. ORFs that were 60 bp long with 40% tBLASTn identity to T4 Hoc or a member of the PKD Ig Pfam were retained. The six contigs containing Ig-like hypervariable domains from the published study by Minot et al. (7) were downloaded from the National Center for Biotechnology Information (NCBI). Identical sequences were collapsed using the Trie clustering method implemented in Qiime (8). The resulting unique sequences were mapped to the position-specific scoring matrix for the PKD Ig Pfam (PF00801) using hmalign (9). The hmalign trimming function was used; sequences that were dominated by gaps after alignment were removed. A maximum likelihood tree was generated from the aligned unique sequences using FastTree version 2.1.1 SSE3 and viewed in MEGA 5. Environmental data for the metagenomes were obtained from the MyMg database. In a separate analysis, structural homology of these same sequences to a carbohydrate-binding protein (10) was determined using the Phyre2 structural homology prediction pipeline (www.sbg.bio.ic.ac.uk/phyre2/html/help.cgi).

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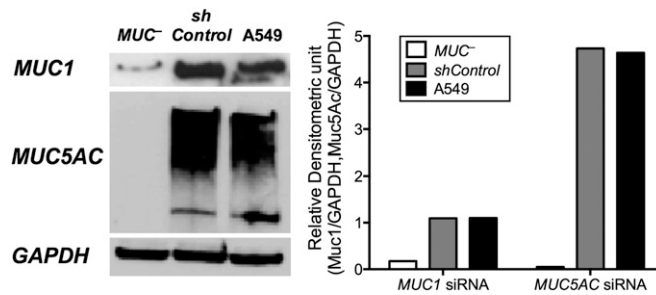


Fig. S4. Western blot analysis of MUC1 and MUC5AC in total cell lysates of A549 lung epithelial cell knockdowns. Lysates of confluent cell layers were separated by SDS/PAGE and then immunoblotted with anti-MUC1 and anti-MUC5AC antibodies. Shown are the MUC⁻ knockdown cell line, the nonsilencing *sh*Control control cell line, and native A549 cells. GAPDH was used as an intracellular protein control.

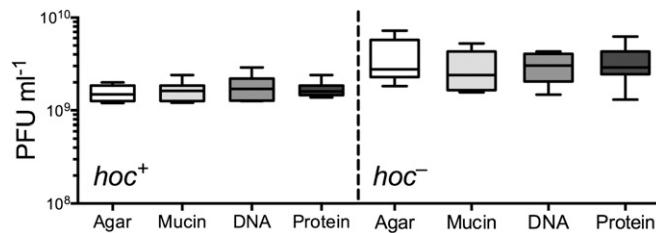


Fig. S5. Surface-free control for the assay of phage adherence to mucus-associated macromolecules. Both *hoc*⁺ and *hoc*⁻ T4 phage (10⁹ pfu·mL⁻¹) were serially diluted to 1 × 10⁻⁷ and 1 × 10⁻⁸, and then incubated in 1% (wt/vol) solutions of mucin, DNA, or protein in 1 mL LB for 30 min at 37 °C. Each incubation mixture was then mixed with *Escherichia coli* top agar and layered over plain agar plates. Resulting plaque-forming unit (PFU) counts showed that infectivity of *hoc*⁺ and *hoc*⁻ T4 phage was not significantly altered in the presence of the macromolecules used in the phage adherence assays (mucin, DNA, and BSA protein).

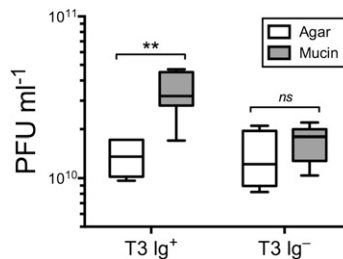


Fig. S6. Adherence of Ig⁺ and Ig⁻ T3 phage to mucin. Phage adherence assays to mucin-coated agar plates were performed as described in *SI Materials and Methods*, except that the Ig⁺ and Ig⁻ T3 phage (10¹¹ pfu·mL⁻¹) were serially diluted to 1 × 10⁻⁹ and 1 × 10⁻¹⁰ pfu·mL⁻¹. The resultant PFU counts of adherent phage showed that Ig⁺ T3 phage adhered to mucin-coated agar plates significantly more than to the plain agar control plates ($n = 6$, $t = 4.443$, $**P = 0.0012$, unpaired t test), whereas there was no significant increase in adherence for the Ig⁻ T3 phage. *ns*, not significant.

Table S2. Glycan microarray analysis of T4 and *hoc*⁻ phage displayed in Fig. 4A

| Glycan no. | Structure | Linkage | T4 RFU | T4 %CV | <i>hoc</i> ⁻ RFU | <i>hoc</i> ⁻ %CV |
|------------|---|------------------------------------|--------|--------|-----------------------------|-----------------------------|
| 609 | GlcNAcb1-3Fuca | -N(CH3)-O-(CH2)2-NH2 | 4,921 | 3 | 394 | 16 |
| 610 | Galb1-3GalNAcb1-4(Neu5Aca2-8Neu5Aca2-8Neu5Aca2-3)Galb1-4Glc | -N(CH3)-O-(CH2)2-NH2 | 4,685 | 11 | 472 | 13 |
| 573 | Neu5Aca2-8Neu5Aca2-3Galb1-3GalNAcb1-4(Neu5Aca2-3)Galb1-4Glc | -N(CH3)-O-(CH2)2-NH2 | 4,161 | 1 | 316 | 11 |
| 608 | Neu5Aca2-6Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-6(Neu5Aca2-6Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4GlcNAcb | Asparagine | 4,685 | 4 | 497 | 17 |
| 145 | Galb1-3GalNAcb1-4Galb1-4Glc | CH2CH2CH2NH2 | 5,823 | 3 | 565 | 7 |
| 195 | Glca1-4Glc | CH2CH2CH2NH2 | 5,845 | 1 | 544 | 3 |
| 514 | GalNAcb1-4(6S)GlcNAc | CH2CH2CH2NH2 | 4,518 | 6 | 568 | 17 |
| 287 | Neu5Gca | CH2CH2CH2NH2 | 4,182 | 3 | 356 | 9 |
| 119 | Gala1-4(Fuca1-2)Galb1-4GlcNAcb | CH2CH2CH2NH2 | 4,157 | 3 | 331 | 8 |
| 336 | GalNAca1-3(Fuca1-2)Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb | CH2CH2NH2 | 7,670 | 31 | 472 | 51 |
| 217 | Manb1-4GlcNAcb | CH2CH2NH2 | 4,766 | 3 | 647 | 24 |
| 144 | Galb1-3GalNAcb1-4(Neu5Aca2-3)Galb1-4Glc | CH2CH2NH2 | 4,797 | 4 | 589 | 26 |
| 517 | Galb1-4(6P)GlcNAcb | CH2CH2NH2 | 4,751 | 11 | 526 | 20 |
| 218 | Neu5Aca2-3Galb1-4GlcNAcb1-3Galb1-4(Fuca1-3)GlcNAcb | CH2CH2NH2 | 4,737 | 3 | 322 | 10 |
| 334 | GalNAcb1-3Gala1-4Galb1-4GlcNAcb1-3Galb1-4Glc | CH2CH2NH2 | 4,261 | 4 | 499 | 8 |
| 143 | Galb1-3GalNAcb1-3Gala1-4Galb1-4Glc | CH2CH2NH2 | 4,236 | 1 | 451 | 13 |
| 581 | Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-6(Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb | EN or NK | 5,465 | 6 | 262 | 7 |
| 360 | Fuca1-2Galb1-3GlcNAcb1-2Mana1-6(Fuca1-2Galb1-3GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4GlcNAcb | GENR | 4,914 | 2 | 482 | 12 |
| 588 | Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-6(Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2)Mana1-6(Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb | KVANKT | 3,926 | 2 | 154 | 5 |
| 470 | Glca1-4Glca1-4Glca1-4Glc | NHCOCH2NH | 7,521 | 10 | 1,288 | 17 |
| 516 | (4S)GalNAcb | NHCOCH2NH | 6,148 | 2 | 464 | 3 |
| 359 | KDNa2-3Galb1-3GalNAca | Threonine (O-linked glycan) | 7,484 | 17 | 1,080 | 24 |
| 471 | Neu5Aca2-3Galb1-4GlcNAcb1-6(Neu5Aca2-3Galb1-4GlcNAcb1-3)GalNAca | Threonine (O-linked glycan) | 5,877 | 6 | 585 | 2 |
| 491 | Neu5Aca2-3Galb1-3GlcNAcb1-6GalNAca | Threonine (O-linked glycan) | 4,755 | 2 | 394 | 8 |
| 596 | Neu5Aca2-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-6(Neu5Aca2-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3)GalNAca | Threonine (O-linked glycan) | 4,703 | 14 | 242 | 7 |
| 595 | GlcNAcb1-3Galb1-4GlcNAcb1-6(GlcNAcb1-3Galb1-4GlcNAcb1-3)GalNAca | Threonine (O-linked glycan) | 4,653 | 1 | 392 | 9 |
| 605 | Neu5Aca2-6Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-6(Neu5Aca2-6Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3)GalNAca | Threonine (O-linked glycan) | 4,078 | 1 | 410 | 10 |
| 480 | Neu5Aca2-6Galb1-4GlcNAcb1-6GalNAca | Threonine (O-linked glycan) | 4,143 | 5 | 301 | 20 |
| 592 | Neu5Aca2-3Galb1-4GlcNAcb1-3Galb1-4GlcNAcb1-3GalNAca | Threonine (O-linked glycan) | 4,200 | 11 | 266 | 11 |

"Glycan no." indicates the glycan ID number used on the Consortium for Functional Glycomics Version 5.1 microarray. "Linkage" denotes the chemical linkage joining the glycan to the macromolecule. Bold threonine linkages represent O-linked glycan residues likely to be associated with mucin glycoproteins.