MICROBIOTA

Gut microbiota utilize immunoglobulin A for mucosal colonization

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The immune system responds vigorously to microbial infection while permitting lifelong colonization by the microbiome. Mechanisms that facilitate the establishment and stability of the gut microbiota remain poorly described. We found that a regulatory system in the prominent human commensal *Bacteroides fragilis* modulates its surface architecture to invite binding of immunoglobulin A (IgA) in mice. Specific immune recognition facilitated bacterial adherence to cultured intestinal epithelial cells and intimate association with the gut mucosal surface in vivo. The IgA response was required for *B. fragilis* (and other commensal species) to occupy a defined mucosal niche that mediates stable colonization of the gut through exclusion of exogenous competitors. Therefore, in addition to its role in pathogen clearance, we propose that IgA responses can be co-opted by the microbiome to engender robust host-microbial symbiosis.

At birth, ecological and evolutionary processes commence to assemble a complex microbial consortium in the animal gut. Community composition of the adult human gut microbiome is remarkably stable during health, despite day-to-day variability in diet and diverse environmental exposures. Instability, or dysbiosis, may be involved in the etiology of a variety of immune, metabolic, and neurologic diseases (*1*, *2*). Longitudinal sequencing studies indicate that a majority of bacterial strains persist within an individual for years (*3*), and for most species there is a single, persistently dominant strain (*4*) (termed "single-strain stability"). Mucus and components of the innate and adaptive immune systems are thought to influence microbiome stability, independently of diet. For example, immunoglobulin A (IgA), the main antibody isotype secreted in the gut, shapes the composition of the intestinal microbiome via currently unknown mechanisms (*5*–*8*). IgA deficiency in mice increases interindividual variability in the microbiome (*9*) and decreases diversity (*10*, *11*). The direct effects of IgA on bacteria have largely been studied in the context of enteric infection by pathogens (*12*). However, early studies of IgA in the healthy gut found that the majority of live bacterial cells in feces are bound by IgA (*13*), reflecting a steady-state IgA response to persistent indigenous microbes (*14*). Studies show that IgA promotes adherence of commensal bacteria to tissue-cultured intestinal epithelial cells (*15*, *16*), although the in vivo implications of this observation are unclear. Furthermore, lack of IgA, the most common human immunodeficiency, does not affect lifespan and only modestly increases susceptibility to respiratory and gastrointestinal infections (*17*); hence, it remains a mystery why the immune system evolved to invest the considerable energy needed to produce several grams of IgA daily (*18*).

Bacteroides fragilis is an important member of the human gut microbiome, with beneficial properties that ameliorate inflammatory and behavioral symptoms in preclinical animal models (*19*–*22*). This commensal exhibits remarkable single-strain stability (*23*, *24*) and enriched colonization of the gut mucosal surface relative to other species (*25*). To explore physical features of *B. fragilis* interaction with the host epithelium, we used transmission electron microscopy (TEM) to visualize colonic tissues of monocolonized mice. *B. fragilis* commonly formed discrete aggregates of tightly packed cells on the apical epithelial surface (Fig. 1A) and penetrated the glycocalyx layer of transmembrane mucins, nearly contacting the microvilli (Fig. 1B and fig. S1, A and B). Intact *B. fragilis* cells were also found nestled in the ducts of the crypts of Lieberkühn (Fig. 1C and fig. S1C). We previously identified a genetic locus in *B. fragilis*, named the commensal colonization factors (*ccfABCDE*), which is necessary for colonization of colonic crypts (*26*). To assess how these genes affect bacterial localization to the mucosal surface, we monocolonized mice with a *ccfCDE* (Δ*ccf*) mutant. TEM showed that *B. fragilis* Δ*ccf* was found only as sparse, individual cells within the epithelial mucosa, excluded from contact with the glycocalyx (Fig. 1, D and E); unlike wild-type *B. fragilis*, it was never observed in aggregates (Fig. 1F). The *B. fragilis* burden in the colon lumen was identical between strains (fig. S2A), which suggests that the CCF system is required specifically for bacterial aggregation within mucus.

High-resolution tomograms of bacterial cells in vivo revealed the presence of a thick, fuzzy capsule layer covering wild-type *B. fragilis* (Fig. 1G), which was significantly reduced in *B. fragilis* Δ*ccf* (Fig. 1, H and I). We sought to investigate the bacterial physiology underlying this ultrastructural change and its potential corresponding effects on colonization. The *ccf* locus is highly induced during gut colonization (*26*) and bacterial growth in mucin O-glycans (*27*), indicating that the CCF system may sense a specific host-derived glycan. The *ccf* genes are homologous to polysaccharide utilization systems in which a sigma factor (*ccfA*) is activated by extracellular glycan sensing (*28*); thus, we hypothesized that *ccfA* may activate genes involved in mucosal colonization. We overexpressed *ccfA* in *B. fragilis* and assessed global gene expression by RNA sequencing (RNA-seq) during in vitro growth [without overexpression, *ccf* is poorly expressed in culture (*26*)]. Of the non-*ccf* genes regulated by *ccfA*, 24 of 25 genes mapped to the biosynthesis loci for capsular polysaccharides A and C (PSA and PSC) (Fig. 2, A and B, and table S1). Correspondingly, *ccf* mutation decreased expression of PSC and increased expression of PSA in vivo (Fig. 2C). Although phase variation of capsular polysaccharides is known to influence the general in vivo fitness of *B. fragilis* (*29*, *30*), these studies identify a pathway for transcriptional regulation of specific polysaccharides in the context of mucosal colonization.

We modeled single-strain stability using a horizontal transmission assay, wherein co-housing animals respectively harboring isogenic strains of wild-type *B. fragilis* resulted in minimal strain transmission from one animal to another (Fig. 2D and fig. S2A). This intraspecies colonization resistance is provided through bacterial occupation of a species-specific nutrient or spatial niche (*26*). However, as previously reported (*26*), when mice were colonized initially with *B. fragilis* Δ*ccf*, animals were permissive to co-colonization by wild-type *B. fragilis* after co-housing (Fig. 2E and fig. S2B), indicating a CCF-dependent defect in niche saturation. Mice harboring a mutant in the biosynthesis genes for PSC (ΔPSC) showed highly variable co-colonization by wild-type bacteria (Fig. 2F and fig. S2C). We observed an unexpected increase in expression of the PSB biosynthesis genes in this mutant (Fig. 2H), which may compensate for the loss of PSC.

We generated a strain defective in synthesizing both PSB and PSC (ΔPSB/C), and mice mono-associated with the double mutant were consistently unable to maintain colonization resistance (Fig. 2G and fig. S2, D to F), even though the strain retained *ccf* expression (fig. S2G). Despite lack of competition in a monocolonized setting and equal levels of colonization in the colon lumen (fig. S2H), the *B. fragilis* Δ*ccf* and ΔPSB/C strains were defective in colonization of the ascending colon mucus (Fig. 2I), reflecting impaired saturation of the mucosal niche.

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Accordingly, when we used TEM to image the ΔPSB/C strain in vivo, although the capsule was not as thin as in *B. fragilis* Δ*ccf* (fig. S2, I and J), the hallmark epithelial aggregation phenotype was abrogated relative to wild-type bacteria (fig. S2, K and L). Therefore, we conclude that the CCF system regulates capsule expression to mediate *B. fragilis* mucosal colonization and single-strain stability.

To investigate host responses contributing to mucosal colonization, we defined the transcriptome of the ascending colon during colonization with wild-type *B. fragilis* or *B. fragilis* Δ*ccf*. Remarkably, 7 of the 14 differentially expressed genes encoded Ig variable chains (Fig. 3A and table S2). We did not observe any elevation of immune responses in Δ*ccf*-colonized mice (fig. S3A), indicating that changes in mucosal association

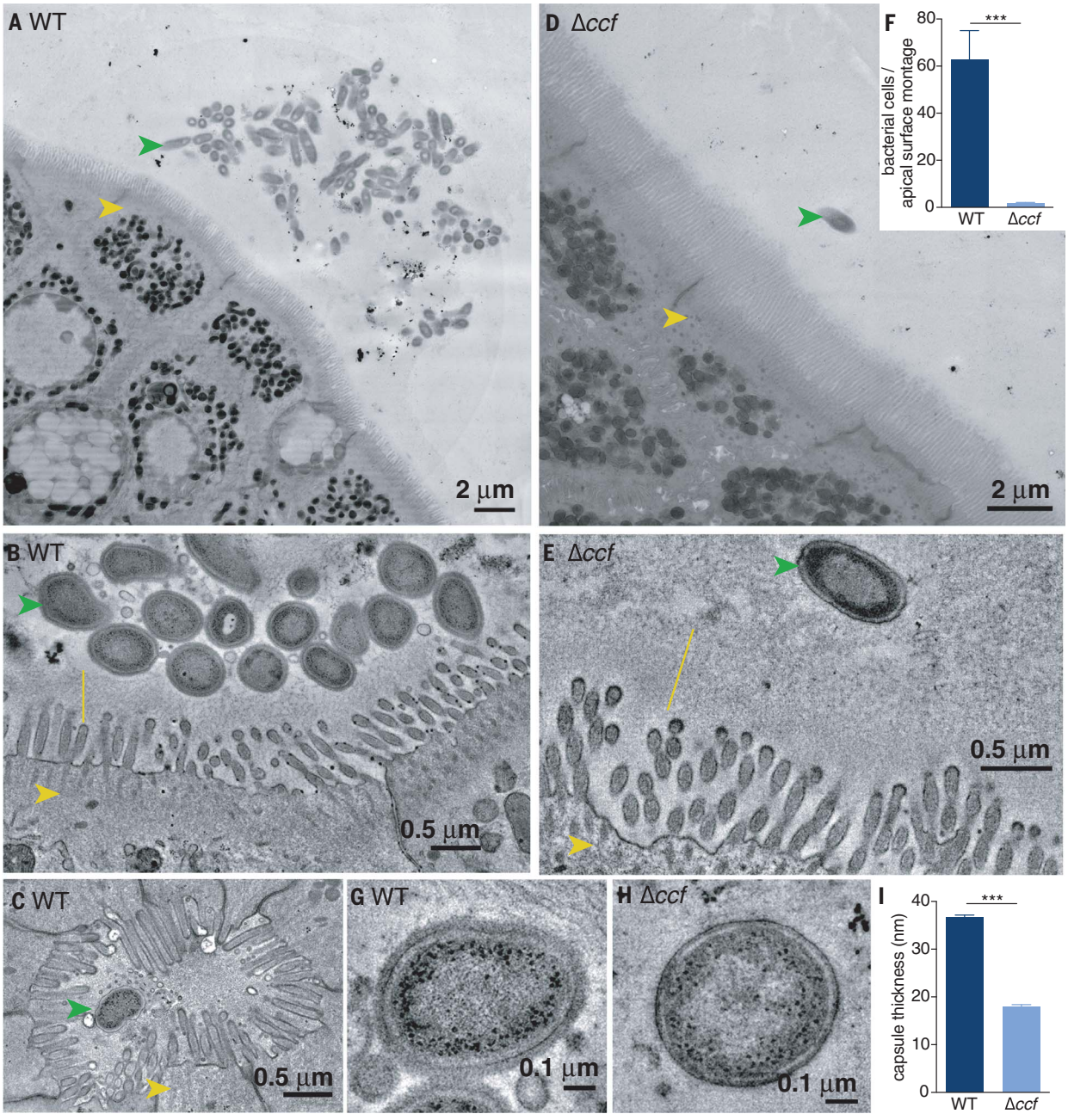

Fig. 1. *Bacteroides fragilis* resides as aggregates on the colon epithelium in a CCF-dependent manner. (**A** and **B**) Representative TEM projection (A) and high-resolution tomogram (B) of epithelial-associated wild-type (WT) *B. fragilis* in monocolonized mice. Under nonpathogenic conditions, ascending colons of mice harbored aggregates of *B. fragilis* (green arrowheads) that made tight associations with the glycocalyx (yellow line) overlying intestinal epithelial cells (yellow arrowheads). (**C**) Tomogram of wild-type *B. fragilis* penetrating deep into the duct of a crypt of Lieberkühn. (**D** and **E**) Representative TEM projection (D) and tomogram (E) of epithelial-associated *B. fragilis* Δ*ccf*. The absence of the CCF system abrogated formation of bacterial aggregates and prevented intimate association with the glycocalyx (n = 3 mice per group, about 1 mm of epithelium scanned per mouse). (**F**) Quantification of bacterial cells per projection montage [(A) and (D)] of epithelial-associated bacteria (mean ± SEM, unpaired t test, n = 7, 8 images from 4 mice per group). (**G** and **H**) Tomograms of the bacterial surface of wild-type *B. fragilis* (G) and *B. fragilis* Δ*ccf* (H) revealed a thick fuzzy capsule for wild-type bacteria residing in the colons of mice. (**I**) Measurement of capsule thickness (mean ± SEM, unpaired t test, n = 10 cells from 3 mice per group). ***$P < 0.001$.

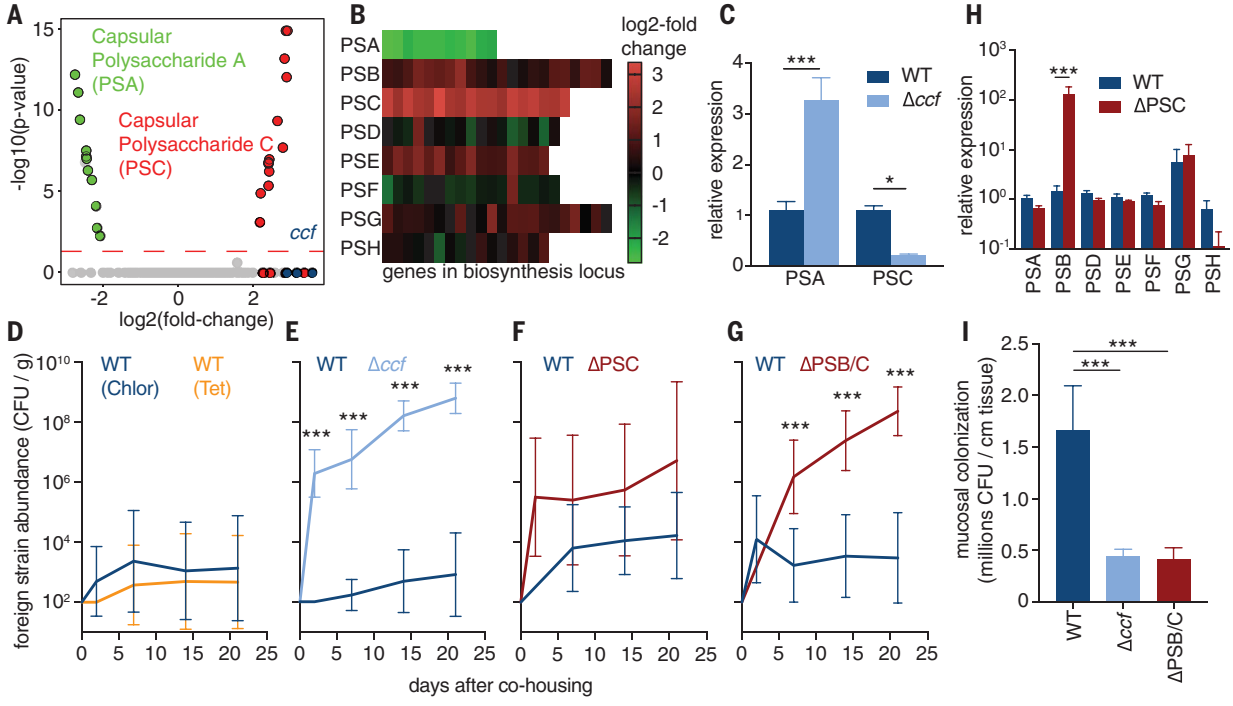

Fig. 2. Specific capsular polysaccharides, regulated by *ccf*, are necessary for single-strain stability. (**A**) RNA-seq gene expression analysis of *B. fragilis* overexpressing *ccfA* during laboratory culture growth, relative to empty vector control (n = 3). Green, PSA genes; red, PSC genes; blue, *ccf* genes. (**B**) Heat map of expression levels for all capsular polysaccharide loci in *B. fragilis* after *ccfA* overexpression during growth in culture. (**C**) Relative expression using quantitative reverse transcription polymerase chain reaction (qRT-PCR; ΔΔCt normalized to gyrase) of RNA from colon lumen contents of mice monocolonized with *B. fragilis* or *B. fragilis* Δ*ccf* [mean ± SEM, Sidak two-way analysis of variance (ANOVA), n = 4]. (**D** to **G**) Abundance of foreign strains exchanged between pairs of co-housed mice each monocolonized with the indicated strains, in colony-forming units (CFU) per gram of feces [Sidak repeated-measures two-way ANOVA on log-transformed data, geometric mean and 95% confidence interval (CI), n = 9 to 12 pairs per plot]. (**H**) Relative expression levels of capsular polysaccharides analyzed by qRT-PCR (ΔΔCt normalized to gyrase) of RNA from colon lumen contents of mice monocolonized with *B. fragilis* or *B. fragilis* ΔPSC (mean ± SEM, Sidak two-way ANOVA, n = 3 for wild type, 4 for ΔPSC). (**I**) Plating of CFU from ascending colon mucus of mice monocolonized with *B. fragilis* strains (mean ± SEM, Tukey ANOVA, n = 8). *P < 0.05, ***P < 0.001.

are not caused by inflammation. Accordingly, we tested whether capsular polysaccharide regulation by *ccf* affects IgA recognition of bacteria (*31*–*33*). In fecal samples from monocolonized animals, wild-type *B. fragilis* was highly coated with IgA, which was significantly diminished in Δ*ccf* and ΔPSB/C strains (Fig. 3, B and C, and fig. S4A). We observed no difference between these strains in the induction of total fecal IgA (Fig. 3D), reflecting equivalent stimulation of nonspecific IgA production (*10*, *34*, *35*). To test bacteria-specific responses, we evaluated IgA that had been extracted from feces of mice monocolonized with *B. fragilis* for binding to bacteria recovered from monocolonized *Rag1*$^{-/-}$ mice (in vivo–adapted, yet IgA-free bacteria). Western blots of bacterial lysates showed that strong IgA reactivity to capsular polysaccharides was abrogated in the Δ*ccf* and ΔPSB/C strains (Fig. 3, E and F). Although IgA can be polyreactive (*10*, *34*, *35*), binding to lysates of *Bacteroides* was species-specific (fig. S4B) and required induction of IgA after bacterial colonization (fig. S4, C and D). Accordingly, in a whole-bacteria binding assay, IgA induced by wild-type bacteria maximally coated wild-type *B. fragilis*, unlike the Δ*ccf* and ΔPSB/C strains (Fig. 3G). IgA induced by *B. fragilis* Δ*ccf* exhibited reduced binding to wild-type bacteria (Fig. 3G). The addition of IgA to in vivo–adapted, IgA-free bacteria increased adherence of *B. fragilis* to intestinal epithelial cells in tissue culture (Fig. 3H), yet had no effect on bacterial viability (fig. S4E). Cell lines known to produce more mucus (*36*) exhibited a greater capacity for IgA-enhanced *B. fragilis* adherence (fig. S4F), consistent with prior work showing that IgA binds mucus (*36*–*38*). IgA-enhanced adherence was decreased whether targeted bacteria lacked *ccf* or PSB/C, or whether the IgA tested was induced by a *ccf* mutant or by *B. thetaiotaomicron* (Fig. 3H and fig. S4G). Whereas pathogenic bacteria elaborate capsular polysaccharides for immune evasion, these results suggest that *B. fragilis* deploys specific capsules for immune attraction, potentially enabling stable mucosal colonization.

We determined whether IgA coating promotes *B. fragilis* colonization in mice. Under the horizontal transmission paradigm, *Rag1*$^{-/-}$ mice colonized with wild-type *B. fragilis* were readily co-colonized by an isogenic strain from a co-housed animal (fig. S5, A and B), showing loss of colonization resistance in the absence of adaptive immunity. We next treated wild-type mice with an antibody to CD20 (fig. S5C) (*39*) to deplete B cells (fig. S5, D to F), thus reducing total fecal IgA levels (fig. S5G) and eliminating IgA coating of wild-type *B. fragilis* during monocolonization (Fig. 4A). IgA recovered from isotype control–treated mice that had been monocolonized with *B. fragilis* promoted adherence of wild-type bacteria to epithelial cells in vitro, whereas IgA from anti-CD20–treated mice had no effect despite being exposed to *B. fragilis* antigens (Fig. 4B). In the horizontal transmission assay, B cell–depleted mice monocolonized with *B. fragilis* were readily invaded by wild-type bacteria, whereas isotype control–injected animals retained colonization resistance (Fig. 4C and fig. S5H). Therefore, active B cell responses to *B. fragilis* colonization enhance single-strain stability.

Because B cell depletion eliminates all antibody isotypes, germ-free IgA$^{-/-}$ mice (*40*) were generated and monocolonized with *B. fragilis*. We did not observe compensatory coating by IgM (fig. S6A). In a horizontal transmission assay with wild-type (BALB/c) and IgA$^{-/-}$ mice, lack of IgA allowed co-colonization by challenge strains (Fig. 4D and fig. S6, B to D), indicating that IgA specifically contributes to single-strain stability. This feature was reproduced in mice with a full microbial community "spiked" with genetically marked *B. fragilis* strains (fig. S6, E and F),

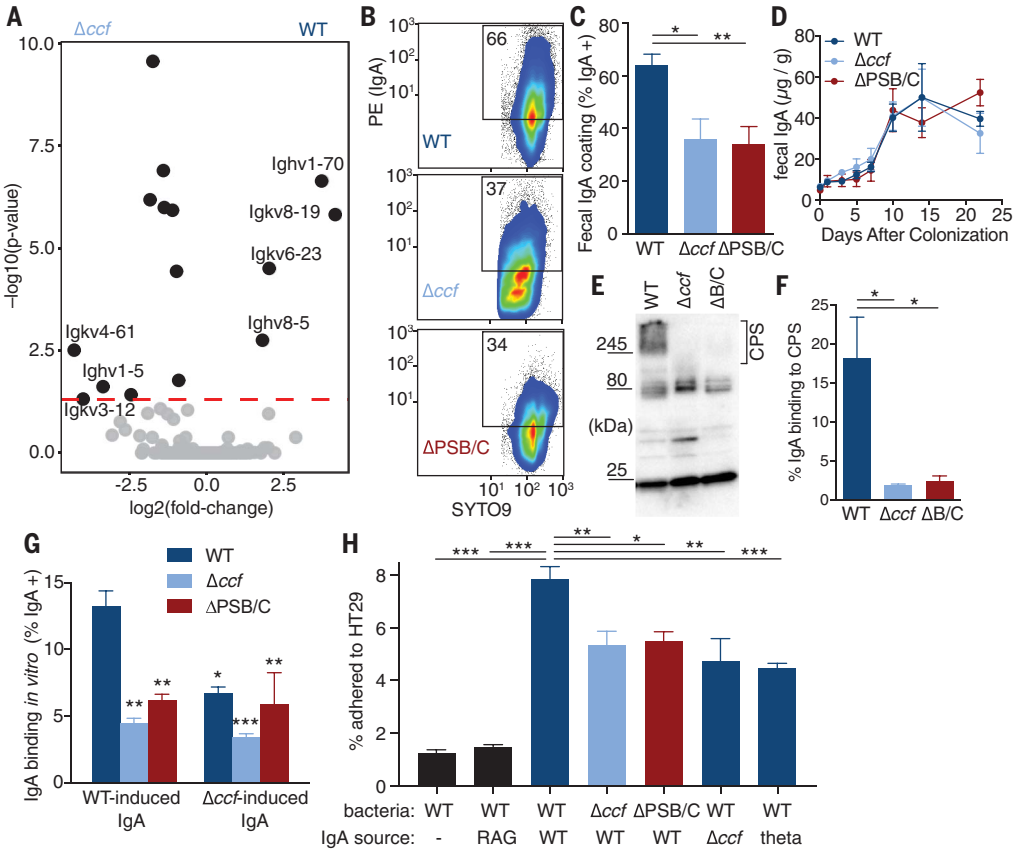

Fig. 3. *B. fragilis* induces a specific IgA response, dependent on *ccf* regulation of surface capsular polysaccharides, which enhances epithelial adherence. (**A**) RNA-seq gene expression analysis of RNA recovered from whole ascending colon tissue of mice monocolonized with *B. fragilis* or *B. fragilis* Δ*ccf* ($n = 3$). (**B**) Flow cytometry plots of *B. fragilis* from feces of monocolonized mice identified with a nuclear stain (SYTO9) and stained with an anti-IgA phycoerythrin (PE)–conjugated antibody. (**C**) Quantification of IgA coating of *B. fragilis* from feces of mice monocolonized with various strains (mean ± SEM, Tukey ANOVA, $n = 11$ or 12). (**D**) Enzyme-linked immunosorbent assay for total fecal IgA in monocolonized mice (mean ± SEM, Sidak repeated-measures two-way ANOVA, not significant, $n = 4$). (**E**) Bacterial lysates from feces of monocolonized *Rag1*$^{-/-}$ mice probed in Western blots with fecal IgA from *B. fragilis* monocolonized mice. (**F**) Quantification of the proportional signal from IgA binding to capsular polysaccharides (CPS) (>245 kDa) (mean ± SEM, Tukey ANOVA, $n = 3$ mice). (**G**) Binding of fecal IgA extracted from monocolonized mice to various strains of *B. fragilis*. Source of IgA is mice colonized with either wild-type *B. fragilis* or *B. fragilis* Δ*ccf*. Because *ccf* is expressed in vivo, IgA-free bacteria from feces of monocolonized *Rag1*$^{-/-}$ mice were used as the target for IgA binding (mean ± SEM, Tukey two-way ANOVA; asterisks denote significant differences from wild-type bacteria with wild-type IgA, $n = 3$). (**H**) In vitro epithelial cell (HT29) adherence assay using IgA extracted from Swiss Webster mice (or *Rag1*$^{-/-}$, second column) monocolonized with *B. fragilis* or *B. thetaiotaomicron* (theta, last column). IgA-free but in vivo–adapted bacteria were isolated from monocolonized *Rag1*$^{-/-}$ mice (mean ± SEM, Tukey ANOVA, $n = 4$ mice as the source of bacteria). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

revealing that single-strain stability of an individual bacterial species occurs in the context of a complex community. Monocolonized IgA$^{-/-}$ mice harbored reduced levels of live bacteria in the colon mucus relative to wild-type mice (Fig. 4E), although they had greater numbers of bacteria in the colon lumen (fig. S6G). TEM images of ascending colon tissues reveal that in IgA$^{-/-}$ animals, wild-type *B. fragilis* failed to aggregate on the epithelial surface (Fig. 4, F and G), similar to the *ccf* and PSB/C mutants in wild-type animals. *B. fragilis* cells also formed aggregates in feces in the presence of IgA (fig. S7), indicating that enhanced mucosal colonization may be due to increased aggregation or growth (*41*) within mucus. These findings converge to support a model whereby *ccf* regulates expression of specific capsular polysaccharides to attract IgA binding, allowing for robust mucosal colonization and single-strain stability.

Beyond *B. fragilis*, we tested whether IgA shapes a complex microbiome after controlled introduction of mouse microbiota to germ-free BALB/c or IgA$^{-/-}$ mice. One month after colonization, despite similar microbiome profiles in feces of both mouse genotypes (fig. S8A), we observed differences for specific taxa (table S3). We also identified a defect in community stratification between the colonic mucus and lumen of IgA$^{-/-}$ mice (Fig. 4H and fig. S8B); this result indicates that IgA is required to individualize microbiome profiles between these two anatomic locations. Remarkably, a highly mucus-enriched exact sequence variant, mapping uniquely to *B. fragilis*, was significantly decreased in the mucus of IgA$^{-/-}$ mice relative to BALB/c mice (Fig. 4I and fig. S9A), naturally supporting our observations from monocolonized mice. To extend this analysis to other microbial species, we identified Rikenellaceae, *Blautia* sp., and segmented filamentous bacteria as being highly IgA-coated (fig. S9B) (*35*), and we assessed the abundance of these taxa in the colonic or ileal mucus. *Blautia* sp. and segmented filamentous bacteria displayed increased mucosal association in the absence of IgA (Fig. 4I) (*42*), demonstrating that IgA can protect the intestinal barrier. However, similar to *B. fragilis*, Rikenellaceae were highly abundant in colon mucus and were significantly depleted in IgA$^{-/-}$ mice (Fig. 4I). We conclude that IgA-enhanced mucosal colonization occurs within complex communities for multiple strains of *B. fragilis* and other species of the gut microbiome.

Classically viewed, the immune system evolved to prevent microbial colonization. However, our findings show that animals tolerate a complex microbiome; moreover, in the case of *B. fragilis*, its intimate association with its mammalian host is (paradoxically) enabled when an immune response is provoked. Related commensal bacteria may also benefit from actively engaging IgA during symbiosis, as *Rag2*$^{-/-}$ mice devoid of adaptive immunity harbor fewer *Bacteroides* (*43*) and both B cell–deficient and IgA$^{-/-}$ animals display decreased colonization by the Bacteroidaceae family (*44*). IgA has been previously shown to increase adherence of *Escherichia coli* (*15*), *Bifidobacterium lactis*, and *Lactobacillus rhamnosus* (*16*) to tissue-cultured epithelial cells, which suggests that these microorganisms may also benefit from IgA to establish a mucosal bacterial community.

Mucosal microbiome instability or loss of immunomodulatory species may underlie the link between IgA deficiency and autoimmune diseases in humans (*45*). Whereas IgA-coated bacteria from individuals with inflammatory bowel disease (*46*) or nutritional deficiencies (*47*) exacerbate respective

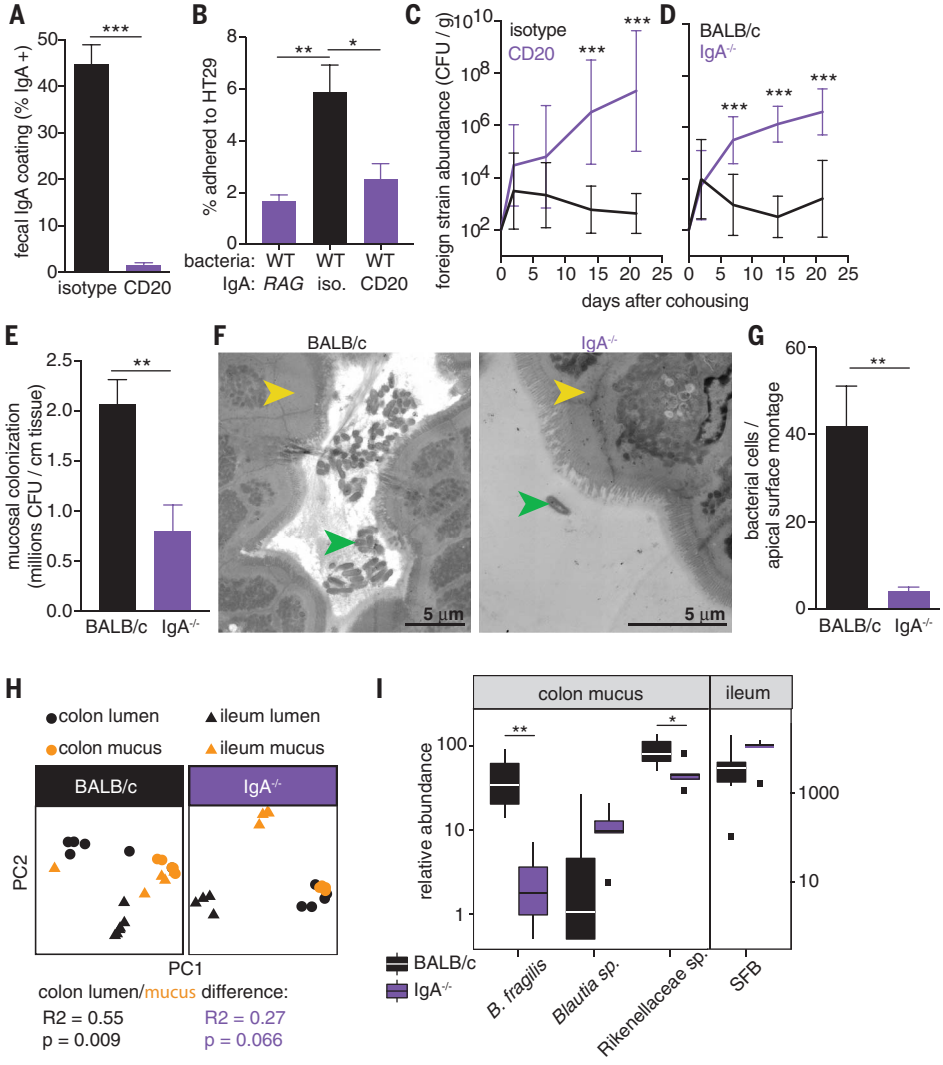

Fig. 4. IgA production in vivo is necessary for single-strain stability, mucosal colonization, and epithelial aggregation. (**A**) IgA coating of wild-type *B. fragilis* in feces after injection of anti-CD20 or isotype control antibody (mean ± SEM, unpaired *t* test, $n = 8$). (**B**) Epithelial cell (HT29) adherence assay of wild-type *B. fragilis* incubated with IgA extracted from the indicated monocolonized mice (mean ± SEM, Tukey ANOVA, $n = 4$ mice as the source of bacteria). (**C**) Abundance of foreign strains exchanged between pairs of wild-type *B. fragilis* monocolonized mice treated with anti-CD20 or an isotype control (geometric mean and 95% CI, Sidak repeated-measures two-way ANOVA on log-transformed data, $n = 10$). (**D**) Foreign strains exchanged between pairs of BALB/c and BALB/c IgA$^{-/-}$ mice monocolonized with wild-type *B. fragilis* (geometric mean and 95% CI, Sidak repeated-measures two-way ANOVA on log-transformed data, $n = 9$). (**E**) CFU plating of ascending colon mucus of wild-type and IgA$^{-/-}$ mice monocolonized with wild-type *B. fragilis* (mean ± SEM, unpaired *t* test, $n = 9$). (**F**) Representative TEM projections of ascending colon (yellow arrowhead: epithelial cell) from mice monocolonized with wild-type *B. fragilis* (green arrowhead) ($n = 3$ mice per group, about 1 mm epithelium scanned per mouse). (**G**) Quantification of bacterial cells per projection montage (mean ± SEM, unpaired *t* test, $n = 7$, 6 images from 3 mice per group). (**H**) Principal coordinates analyses of weighed UniFrac distances of 16*S* community profiles of ex–germ-free BALB/c and BALB/c IgA$^{-/-}$ mice transplanted with a complex mouse microbiota (Adonis test within colon for lumen/mucus difference). (**I**) Relative abundance of *B. fragilis* and highly IgA-coated exact sequence variants in ex–germ-free mice. SFB, segmented filamentous bacteria (median and interquartile range). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

pathologies in mice, IgA-coated bacteria from healthy humans protect mice from disease (*47*). We propose that during health, IgA fosters mucosal colonization of microbiota with beneficial properties (*9*), whereas disease states may induce (or be caused by) IgA responses to pathogens or pathobionts that disrupt healthy microbiome equilibria. Indeed, computational models indicate that IgA can both maintain indigenous mucosal populations and clear invasive pathogens (*48*). In addition to serving as a defense system, adaptive immunity apparently evolved to engender intimate association with members of the gut microbiome.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/360/6390/795/suppl/DC1
Materials and Methods
Figs. S1 to S9
Tables S1 to S5
References (*49*–*69*)

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Gut microbiota utilize immunoglobulin A for mucosal colonization

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Benign colonization of the gut

Microbial communities in the gut can be highly individual. What engenders this specificity? The gut characteristically produces gram quantities of immunoglobulin A (IgA) antibody, which is presumed to protect the gut from pathogen attack. Donaldson *et al.* engineered strains of *Bacteroides fragilis*, a common human commensal, to modify its surface capsule, which affects its ability to colonize the germ-free mouse gut. Capsule changes altered the capacity of IgA to bind to the different mutants. It seems that this commensal species exploits IgA sticking power specifically to give it a competitive edge and to promote its establishment in the gut.

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