Cell Host & Microbe
Article

IgA Response to Symbiotic Bacteria as a Mediator of Gut Homeostasis

Daniel A. Peterson,[1,2] Nathan P. McNulty,[2] Janaki L. Guruge,[2] and Jeffrey I. Gordon[2,*]
[1]Department of Pathology and Immunology
[2]Center for Genome Sciences
Washington University School of Medicine, St. Louis, MO, 63108 USA
*Correspondence: jgordon@wustl.edu
DOI 10.1016/j.chom.2007.09.013

SUMMARY

Colonization of germ-free mice with a normal gut microbiota elicits bacteria-specific IgA antibody responses. The effects of these responses on microbial and host biology remain poorly defined. Therefore, we developed a gnotobiotic mouse model where the microbiota is reduced to one bacterial species, and the antibody repertoire to a single, monoclonal IgA against the bacterium's capsular polysaccharide. *Bacteroides thetaiotaomicron* was introduced into germ-free wild-type, immunodeficient *Rag1*$^{-/-}$, or *Rag1*$^{-/-}$ mice harboring IgA-producing hybridoma cells. Without IgA, *B. thetaiotaomicron* elicits a more robust innate immune response and reacts to this response by inducing genes that metabolize host oxidative products. IgA reduces intestinal proinflammatory signaling and bacterial epitope expression, thereby balancing suppression of the oxidative burst with the antibody's negative impact on bacterial fitness. These results underscore the adaptive immune system's critical role in establishing a sustainable host-microbial relationship. Immunoselection of bacterial epitope expression may contribute to the remarkable strain-level diversity in this ecosystem.

INTRODUCTION

Our adult bodies are home to trillions of microbes, producing a "supraorganism" whose microbial cell population exceeds the number of human cells by an estimated order of magnitude. Our gut contains the vast majority of our bacterial and archaeal partners (Ley et al., 2006a). Over 90% of the bacterial phylogenetic types (phylotypes) belong to just two divisions (superkingdoms)—the Bacteroidetes and the Firmicutes. Each of us appears to harbor a distinctive collection of species- and strain-level phylotypes belonging to these two dominant bacterial divisions. The presence of relatively few bacterial and archaeal divisions in this environmental ecosystem indicates that strong selective pressures operate to shape the community (Ley et al., 2006a).

Deciphering how an individual's immune system and microbiota coevolve should help provide answers to a number of intriguing questions. How is our microbiota selected? How does it manifest compositional diversity and functional stability? How does it adapt to changes in our lifestyles? How do perturbations in microbial ecology contribute to certain pathologic states such as infectious diarrheas, inflammatory bowel diseases, and metabolic abnormalities, for example (Frank et al., 2007; Ley et al., 2006b; Turnbaugh et al., 2006)? How can the representation of components of our microbiota be intentionally manipulated for therapeutic benefit?

Changes in gut microbial ecology have been documented in mice with immune deficiencies (Fagarasan et al., 2002; Suzuki et al., 2004). Colonization of germ-free mice with members of the normal gut microbiota elicits bacteria-specific IgA responses (Shroff et al., 1995). In the few reports where the specificities of these antibodies have been determined, their effects on microbial and host biology have not been described (Macpherson et al., 2000). However, when immunodeficient *Rag1*$^{-/-}$ or SCID mice, which lack a functional adaptive immune system, are colonized with single or multiple species of bacteria, they display a more robust *innate* immune response than their immunocompetent wild-type counterparts (Keilbaugh et al., 2005; Cash et al., 2006). This indicates that in normal mice the adaptive immune response plays a critical role in minimizing activation of the innate immune system by the gut microbiota. In models of T cell-mediated colitis produced either by adoptive transfer of CD45RB^{high} T cells into SCID recipients or by gene knockouts, immunoglobulin (Ig) impacts mucosal inflammation (Gerth et al., 2004; Kanai et al., 2006). Moreover, serum responses to microbial antigens are associated with inflammatory bowel disease in mice and humans (Landers et al., 2002), as are defects in the innate and adaptive immune system (Cobb et al., 2006; Izcue et al., 2006).

Based on these observations, we developed a gnotobiotic mouse model to define mechanisms by which an IgA response plays a key role in establishing and maintaining a noninflammatory host-microbial relationship. In this model, the diversity of the gut microbiota is reduced to a single prominent bacterial member of the human distal intestinal ecosystem, and the repertoire of the adaptive

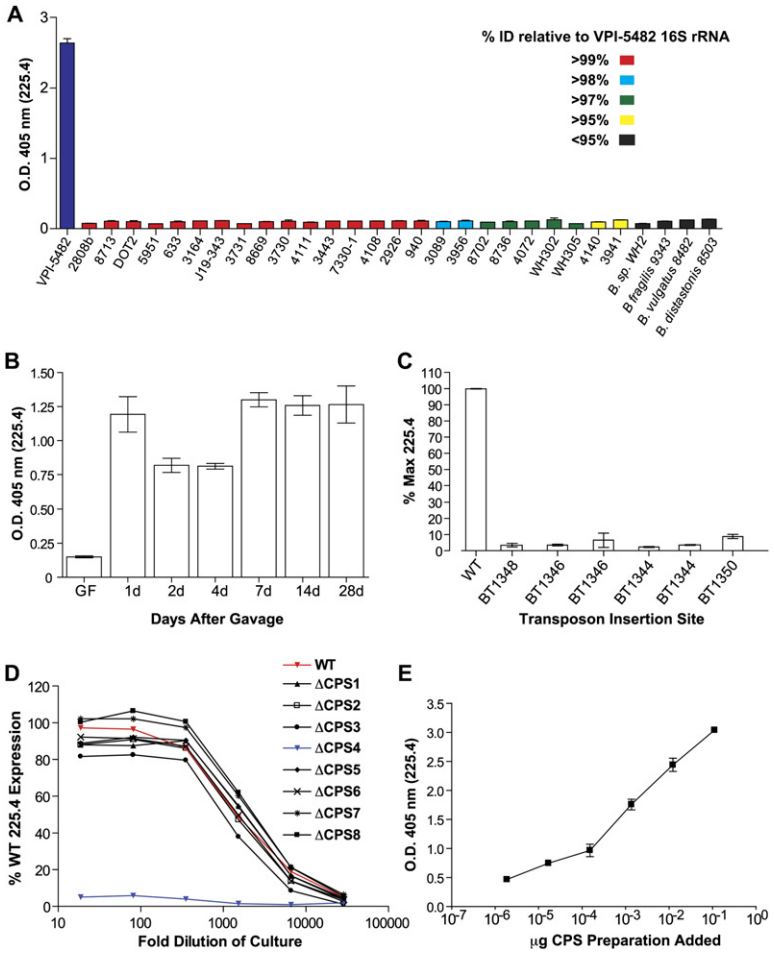


Figure 1. Characterization of the Epitope Recognized by the 225.4 Monoclonal IgA Antibody

(A) ELISA reveals that the antibody reacts only with the type strain (VPI-5482) and not with any other *B. thetaiotaomicron* isolates or four other sequenced human gut-associated Bacteroides species. Colors indicate the degree of phylogenetic relatedness to *B. thetaiotaomicron* VPI-5482 (defined by percent 16S rRNA nucleotide sequence identity using ARB [http://www.arb-home.de/]).

(B) Expression of the 225.4 epitope in fecal pellets obtained from germ-free wild-type B6 mice colonized for the indicated periods of time. ELISAs were performed using biotinylated 225.4.

(C) ELISA assays of transposon mutants with diminished or absent 225.4 reactivity (*B. thetaiotaomicron* [BT] gene name given at site of transposon insertion).

(D) Reactivity of 225.4 mAb with isogenic *B. thetaiotaomicron* strains containing pGERM disruptions of *CPS* locus expression. Mutants were all grown in TYG medium to stationary phase: aliquots, diluted in bicarbonate buffer, were used to coat ELISA plates. 225.4 recognized all but the *CPS4* mutant.

(E) Dose-response ELISA of a preparation of the 225.4 epitope purified from *B. thetaiotaomicron* using the hot water-phenol method typically employed to isolate capsular polysaccharides. Prior to the assay, lipopolysaccharide (LPS) was removed by ultracentrifugation.

immune system is reduced to one naturally primed Ig directed against an identified capsular polysaccharide epitope expressed by the bacterium in vivo.

RESULTS

We chose *B. thetaiotaomicron* as a model symbiont for our experiments. It possesses a large arsenal of glycoside hydrolases for breaking down dietary polysaccharides that our own human proteome is ill-equipped to process (Sonnenburg et al., 2005) and efficiently colonizes the intestines of adult germ-free C57Bl/6J mice. Twenty-four hours after gavage with 10^8 CFU of the sequenced *B. thetaiotaomicron* type strain, VPI-5482, bacteria achieve a density in the small intestine and cecum that does not change significantly over the ensuing 6 months (Figure S1). ELISA of serum levels of IgG subtypes, IgM and IgA 1 day, 2 days, 4 days, 7 days, 14 days, 28 days, and 6 months after gavage of germ-free recipients disclosed that the IgG2a subtype exhibited the greatest relative increase (75-fold), while IgG1 changed $\leq$1.5-fold (Figure S2), consistent with a CD4 T-helper 1 (Th1) environment (Mazmanian et al., 2005). These observations led us to predict that *B. thetaiotaomicron* reactive B cells would be present within the intestinal wall at the 14 day time point and could be captured by hybridoma fusion, thereby allowing us to immortalize a single naturally primed, bacterial epitope-specific IgA response (see Experimental Procedures for additional details).

B. thetaiotaomicron-primed IgA-producing hybridomas were identified (see Experimental Procedures), including one that produced a mAb, named 225.4, specific for the sequenced VPI-5482 type strain. ELISA disclosed that this mAb does not react with bacteria closely related to *B. thetaiotaomicron*, or with any of 1200 bacterial colonies recovered from the ceca of conventionally raised B6 mice and feces of healthy human donors (Figure 1A and data not shown). Using biotinylated 225.4 mAb, we tested fecal pellets for expression of the epitope and found that it was produced by the bacterium in vivo starting from day one of colonization (Figure 1B).

With a finished genome sequence available for *B. thetaiotaomicron* VPI-5482 (Xu et al., 2003), we were able to identify genes required for generation of the 225.4 epitope. A library of 4600 transposon (Tn4351) mutants was generated (see Experimental Procedures for details) and screened by ELISA for colonies that had lost their 225.4 reactivity (Figure 1C). Sixty-five percent of the 225.4-negative mutants contained inserts in the capsular polysaccharide synthesis 4 (*CPS4*) locus; three of the

Table 1. Tn4351 Insertion Sites Associated with Markedly Diminished or Absent 225.4 Epitope Expression in *B. thetaiotaomicron*

Insertion Site	Gene	Gene Description	Insertion Site	Gene	Gene Description
→		100 base pairs upstream of BT1358	→	BT4576	hypothetical protein
	BT1358	UpxZ homolog		BT4575	hypothetical protein
	BT1357	UpxY homolog			
→	BT1356	polysialic acid transport protein kpsD precursor	→	BT2642	conserved hypothetical protein
→	BT1355	polysaccharide biosynthesis protein chain length determinator		BT2643	conserved hypothetical protein
	BT1354	flippase		BT2644	DNA topoisomerase I
→	BT1353	glycosyltransferase (GT 2)			
	BT1352	glycosyltransferase (GT 4)			
	BT1351	glucose-1-phosphate cytidylyltransferase	→	BT2952	SusC homolog
→	BT1350	CDP-glucose 4,6-dehydratase		BT2951	SusD homolog
	BT1349	dTDP-4-dehydrorhamnose 3,5-epimerase		BT2950	hypothetical protein
	BT1348	CDP-abequose synthase		BT2949	α-1,6-mannanase
	BT1347	glycosyltransferase (GT 2)		BT2948	α-1,2-mannosidase
*→	BT1346	capA domain protein			
	BT1345	glycosyltransferase	→	BT3775	mannosyltransferase
*→	BT1344	glycosyltransferase (GT 4)			
	BT1343	capsule biosynthesis protein	→	BT1035	β hexaminidase
	BT1342	UDP-glucuronic epimerase		BT1034	putative signal transducer
	BT1341	UDP-glucose 6-dehydrogenase		BT1033	hypothetical protein
	BT1340	lipopolysaccharide biosynthesis glycosyltransferase (GT 2)		BT1032	α-1,2-mannosidase
	BT1339	undecaprenyl-phosphate acetylglucosaminyltransferase			
	BT1338	DTDP-4-dehydrorhamnose 3,5-epimerase	→	BT0664	ABC transporter substrate-binding protein
				BT0665	hypothetical protein
				BT0666	hypothetical protein
				BT0667	signal peptidase I
				BT0668	glutathione synthetase

Arrows indicate the gene that contains the transposon insertion. Downstream genes in known or predicted operons are also listed (Westover et al., 2005).
* Indicates two unique mutants were identified in this gene. Glycosyltransferases (GT) are classified based on their assignment to families in the Carbohydrate-Active Enzymes (CAZy) database (http://www.cazy.org/).

other six loci are known or predicted to be related to various aspects of carbohydrate metabolism (Table 1).

To confirm the *CPS4* dependence of the 225.4 epitope, we disrupted all eight of the bacterium's *CPS* loci using a suicide vector. Each of the resulting isogenic strains harbored a polar insertion in the first gene of each locus. When analyzed by ELISA, only the *CPS4* disruption was associated with loss of epitope (Figure 1D). Hot water-phenol extraction of wild-type bacteria recovered after overnight growth in rich TYG medium revealed that 225.4 reactivity was retained in the water phase of the extract (Figure 1E). In addition, the epitope was resistant to digestion with DNase, RNase, and proteases, but susceptible to acid hydrolysis (data not shown). Thus, a combination of biochemical and genetic data are consistent with the notion that 225.4 recognizes a surface capsular

carbohydrate epitope whose production is directed by the *CPS4* locus and influenced by additional genes distributed throughout the genome. The exquisite strain specificity of the antibody is reminiscent of strain-specific responses to encapsulated pathogens such as *S. pneumoniae* (Coughlin et al., 1998). While it is unclear how well the 225.4 specificity is represented in the normal anti-*B. thetaiotaomicron* repertoire, our results indicate that it is clearly a member of a naturally primed immune response.

The next question we addressed was the impact of this specific antisymbiont IgA in vivo. Therefore, germ-free *Rag1*$^{-/-}$ mice, which lack mature T and B cells, were injected with 225.4-producing hybridoma cells under their dorsal skin, creating a hybridoma "backpack" mouse where the only Ig in the serum and gut lumen was 225.4 (Michetti et al., 1992). We waited 10 days before colonizing these backpack animals with wild-type *B. thetaiotaomicron*: this decision was based on an analysis of the time course of rise of serum 225.4 mAb levels in *Rag1*$^{-/-}$ backpack controls (Figure S3A). Variations in levels of 225.4 IgA in the sera of different animals at this time point were linked to differences in the growth of their backpack hybridomas and correlated with levels of antibody excreted into their intestinal lumen ($r^2 = 0.94$ based on ELISA of fecal pellets; n = 12 mice; Figure S3B). This feature allowed us to conduct a dose-response study of the effects of the capsular epitope-specific IgA on the host-bacterial relationship.

The first in vivo effect of the 225.4 antibody response that we measured was on expression of its own epitope. Ten days after colonization of germ-free *Rag1*$^{-/-}$ mice with implanted backpacks, and *Rag1*$^{-/-}$ controls with no backpacks (n = 4–16/group, 2 replicate experiments), animals were sacrificed, and hot water-phenol extracts of their cecal contents were prepared. ELISA revealed that (1) levels of the 225.4 antigen were significantly decreased in the ceca of *Rag1*$^{-/-}$ backpack mice even though there were no significant differences in bacterial density between the two groups of animals ($p < 0.01$; Figures 2A and 2B) and (2) there was an inverse relationship between antibody levels and luminal levels of its epitope: i.e., lowest levels of epitope were documented in mice with the greatest concentration of 225.4 IgA ($r^2 = 0.73$; Figure 2C). qRT-PCR assays of RNA prepared from cecal contents confirmed significantly decreased expression of the bacterial *CPS4* locus in the presence of the 225.4 antibody ($p < 0.05$; Figure 2D).

The second effect of the antibody response we measured was its impact on bacterial fitness. Colony-forming unit (CFU) and ELISA assays of cecal contents, harvested from gnotobiotic *Rag1*$^{-/-}$ mice with and without backpacks 10 days after cocolonization with equivalent numbers of the isogenic wild-type and Δ*CPS4* mutant strains, established that, in the absence of any 225.4 antibody, the representation of the mutant in this habitat was 100- to 1000-fold lower than wild-type (Figures 3A–3C). The basis of the fitness defect produced by *ΔCPS4* remains unknown.

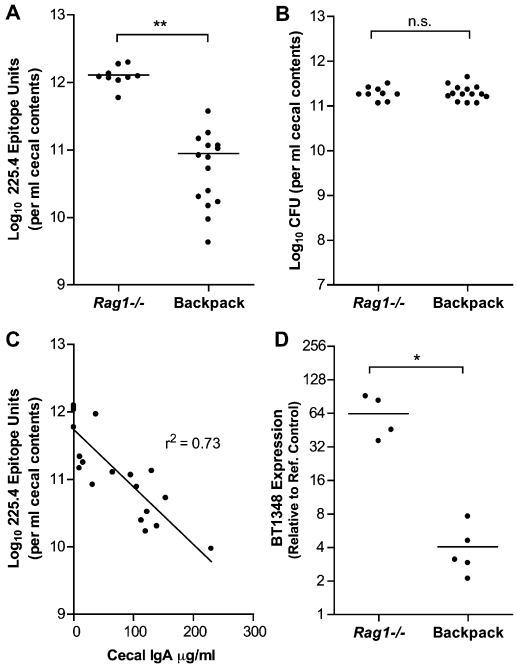


Figure 2. Impact of 225.4 IgA In Vivo

Adult germ-free *Rag1*$^{-/-}$ mice, with or without 225.4 mAb-producing backpack hybridomas, were colonized for 10 days with wild-type *B. thetaiotaomicron*.

(A) Concentration of 225.4 epitope in cecal contents (see Experimental Procedures).

(B) Density of colonization of the ceca of gnotobiotic mice.

(C) Correlation between 225.4 epitope levels and 225.4 IgA levels in cecal contents.

(D) qRT-PCR assays of RNA prepared from cecal contents. Expression of BT1348, a member of the *CPS4* locus, was used as a biomarker for locus expression. Data were normalized to 16S rRNA (for each sample) and expressed as fold difference of each mouse compared to a *B. thetaioatomicron*-colonized wild-type C57Bl/6J (B6) reference control ($\Delta\Delta C_T$ method). *$p < 0.05$; **$p < 0.01$.

The ratio of wild-type to isogenic *ΔCPS4* mutant cells was shifted by an order of magnitude in favor of the mutant in mice with high levels of cecal 225.4 antibody, reflecting a reduction in the dominance of the wild-type bacterium (the representation of the wild-type strain decreased from 99.9% to 99% of the population; Figure 3C). In mice with <5 μg (0.4–2.3 μg) of 225.4 antibody per ml of cecal contents, *B. thetaiotaomicron* 225.4 epitope expression was still significantly reduced compared to *Rag1*$^{-/-}$ animals without backpacks (Figure 3D), but bacterial fitness was not detectably reduced as judged by the ratio of wild-type to *ΔCPS4* mutant CFUs in their ceca (Figure 3C).

We next examined the in vivo effect of the 225.4 antibody on global bacterial gene expression. Whole-genome transcriptional profiling with custom *B. thetaiotaomicron* GeneChips containing probe sets that cover 98.6% of the bacterium's 4779 protein-coding genes (Sonnenburg et al., 2005) was used to define the impact of this engineered antisymbiont immune response on bacterial physiology in vivo. We compared wild-type *B. thetaiotaomicron*

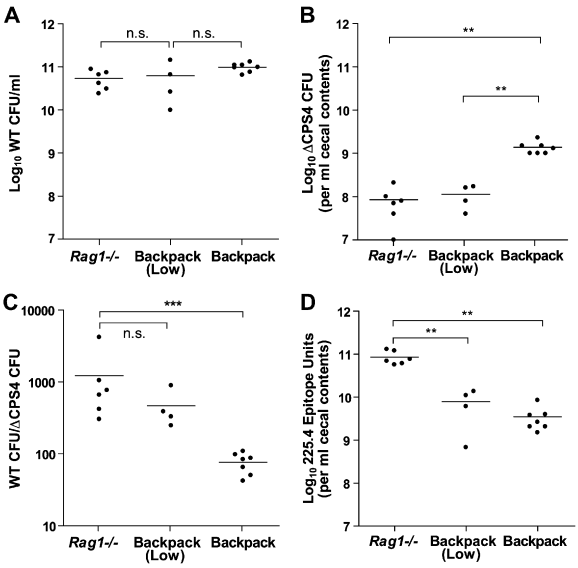


Figure 3. The Impact of Naturally Primed 225.4 Antibody on Competition In Vivo

Germ-free *Rag1*$^{-/-}$ mice, with or without 225.4-producing hybridoma backpacks, were colonized with a 1:1 mixture of the *B. thetaiotaomicron* type strain (VPI-5482) and an isogenic *CPS4* null mutant (Δ*CPS4*; erythromycin-resistant). Cecal contents were harvested 10 days later, and the representation of the two strains was defined by plating on medium with and without erythromycin.

(A) CFU of wild-type strain in *Rag1*$^{-/-}$ animals, *Rag1*$^{-/-}$ mice plus backpack (range of IgA levels, 5.75–175 μg/ml cecal contents; geometric mean, 18.6 μg/ml), or "backpack-low" animals (cecal IgA, 0.4–2.3 μg/ml; geometric mean, 0.9 μg/ml). n.s., no significant difference.

(B) The less competitive Δ*CPS4* mutant demonstrates a 10-fold increase in density in backpack mice with cecal IgA > 5 μg/ml compared to *Rag1*$^{-/-}$ controls or *Rag1*$^{-/-}$ "backpack-low" animals.

(C) Ratio of wild-type to Δ*CPS4* bacterial CFU/ml of cecal contents in *Rag1*$^{-/-}$, *Rag1*$^{-/-}$ backpack, or *Rag1*$^{-/-}$ "backpack-low" mice. The competitive advantage of wild-type *B. thetaiotaomicron* decreases nearly 10-fold in backpack mice with cecal IgA > 5 μg/ml.

(D) 225.4 epitope expression the ceca of *Rag1*$^{-/-}$ mice without backpacks, and *Rag1*$^{-/-}$ mice with high and low levels of cecal IgA. Levels of epitope are significantly decreased in backpack mice. Wild-type B6 mice (polyclonal IgA control) have the same levels of wild-type and Δ*CPS4* CFU, and the same 225.4 epitope levels, as *Rag1*$^{-/-}$ controls ($p > 0.05$; data not shown). **$p < 0.01$ ***$p < 0.001$ according to Student's t test or ANOVA, as appropriate.

recovered from the cecum after a 10 day colonization of *Rag1*$^{-/-}$ mice without backpacks (reference control, no antibody response), with bacteria harvested from *Rag1*$^{-/-}$ backpack animals (monoclonal antibody response to a single surface capsular epitope) and wild-type B6 mice minus backpacks (polyclonal antibody response to multiple *B. thetaiotaomicron* epitopes) (n = 4 mice/group; each cecal sample analyzed separately with its own bacterial GeneChip) (see Figure S4 for the results of unsupervised hierarchical clustering of GeneChip data sets and Table S1 for an annotated list of differentially expressed genes).

Notably, in the absence of an immune response (i.e., *Rag1*$^{-/-}$ mice without backpacks), there was significantly higher expression of (1) an operon (BT1414-1418) that encodes nitrite reductase, (2) another gene (BT0687) involved in nitric oxide metabolism (Rodionov et al., 2005), and (3) an operon encoding subunits of cytochrome D ubiquinol oxidase (implicated in aerotolerance) (Figure 4A).

Inducible nitric oxide synthase (iNOS) is a prototypic member of the host's oxidative response pathway, and nitric oxide is an evolutionarily conserved component of the innate immune response (Davidson et al., 2004). qRT-PCR assays of RNA prepared from the distal small intestines of mice belonging to the three different groups established that iNOS expression was, on average, 5-fold higher in *Rag1*$^{-/-}$ mice without backpacks compared to *Rag1*$^{-/-}$ mice with backpacks, and 23-fold higher compared to wild-type B6 animals ($p < 0.01$; Figure 4B). Moreover, in *Rag1*$^{-/-}$ mice with and without backpack tumors there was a direct and significant correlation between levels of expression of BT1417 (nitrite reductase locus member) and iNOS ($r^2 = 0.75$; Figures 4C and 4D).

These results indicate that, in the absence of a secreted IgA, *B. thetaiotaomicron* elicits a more robust oxidative response in the host and adapts by inducing bacterial genes involved in the metabolism of products of the host response. They provide evidence that a quiescent relationship between *B. thetaiotaomicron* and its host is predicated on the Ig(A) response and are consistent with a model where an iterative set of adaptations involving symbiont and host results in a coevolved homeostasis. In both *Rag1*$^{-/-}$ and wild-type gnotobiotic mice, possessing CPS4 gives the bacteria a competitive advantage over isogenic strains that lack it. There appears to be an "optimal" level of anti-CPS response: at high levels of 225.4 IgA, CPS4-associated epitope expression is decreased, but not extinguished (Figures 2A–2C), and innate cell activation is diminished; however, if epitope levels decrease too much under immune pressure *B. thetaiotaomicron* may elicit a more robust innate immune response (as suggested by Figure 4E).

The final effect of the antisymbiont antibody response examined was its impact on intestinal gene expression. Transcriptional profiling of distal small intestinal RNA prepared from the same mice as those described above revealed that, in addition to iNOS, wild-type *B. thetaiotaomicron*-colonized *Rag1*$^{-/-}$ animals without backpacks exhibited a marked upregulation of other genes involved in innate inflammatory responses when compared to colonized *Rag1*$^{-/-}$ mice with backpacks or wild-type B6 animals (n = 3–5/group; see Figure S5 and Table S2 for gene lists). Ingenuity Pathway Analysis (IPA) software was subsequently used to organize these regulated genes, as well as genes that are expressed but not regulated, into known interaction networks. A large network composed of complement components, phospholipase A2 group 2A (PLA2G2A), regenerating islet-derived 1-β (Reg1-β), interleukin-1 receptor-antagonist (IL1-RN), and iNOS (with concomitant decreases in expression of arginase 2, a competitive iNOS inhibitor) is shown in Figure 5 and Tables S3 and S4. Downstream signaling pathways are also upregulated in *Rag1*$^{-/-}$ mice without backpacks

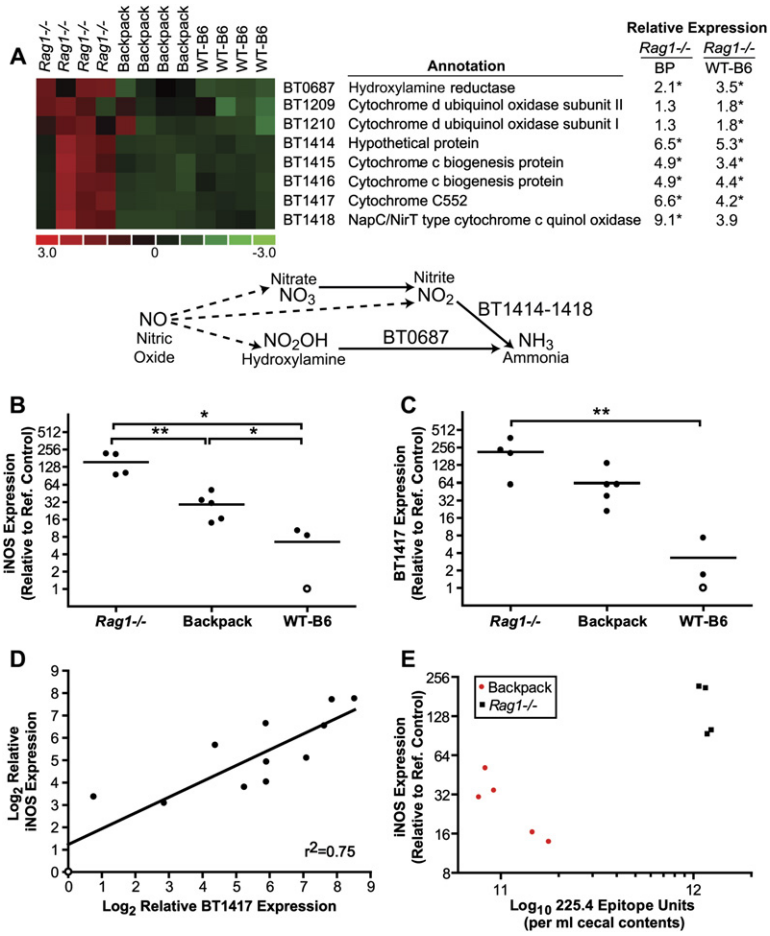


Figure 4. Functional Genomics Analysis of the Effects of 225.4 IgA on Bacterial and Host Gene Expression

(A) *B. thetaiotaomicron* GeneChip analysis of bacterial genes, known or predicted to be involved in oxidative stress responses, that are modulated by the presence of an adaptive immune response (i.e., whose expression was significantly different when comparing *Rag1*$^{-/-}$ to backpack or B6 mice). Standard deviations above (increasing red) or below (increasing green) the mean level of expression (black) of a gene across all animals are indicated; *$p < 0.05$ according to Student's t test. Pathways for nitric oxide (NO) metabolism are shown.

(B–E) qRT-PCR analysis of mouse iNOS (NOS2a) and bacterial nitrite reductase (BT1417) expression in vivo. The relative expression in each sample was compared to a reference mouse in the wild-type B6 control group (indicated by open circle). (B) RNA isolated from the distal small intestines of mice colonized for 10 days with wild-type *B. thetaiotaomicron*. *Rag1*$^{-/-}$ mice without backpacks have significantly higher levels of iNOS expression than wild-type B6 controls. Backpack *Rag1*$^{-/-}$ mice (range of cecal IgA, 9–153 μg/ml; geometric mean, 38 μg/ml) display a significant decrease in iNOS expression compared to *Rag1*$^{-/-}$ controls. (C) BT1417, a gene in a nitrite reductase operon, demonstrates a similar pattern of downregulation by 225.4 IgA in vivo. (D) Direct relationship between BT1417 and iNOS expression. (E) Inverse relationship between cecal 225.4 epitope levels and iNOS expression. *$p < 0.05$; **$p < 0.01$.

compared to controls, including signal transducer and activator of transcription-3 (STAT3) and STAT6, and interferon regulatory factor-8. In addition, other known markers of innate immune cell activation not in this network are affected, such as chemokine CX3CL1 (Fractalkine), nuclear factor κB (NFκB), and Reg3-γ. Since *Rag1*$^{-/-}$ mice lack B and T lymphocytes, these transcriptional profiles reflect activation of genes in intestinal epithelial cells (including members of the Paneth lineage), natural killer cells, and/or macrophages/dendritic cells (DC) that are present in these animals.

Two observations emphasize that the impact of the engineered 225.4 IgA response on the host-microbial relationship is the result of recognition of a bacterial surface epitope, rather than a unique property of the *CPS4* epitope per se. Colonization of germ-free *Rag1*$^{-/-}$ mice without backpacks (no antibody response) and wild-type B6 mice with the Δ*CPS4* strain produced the same effects on iNOS expression as the wild-type strain (Figure 4 and Figure S6). Moreover, in the wild-type B6 mice, where 225.4 specificity is only one component of the adaptive polyclonal IgA response to colonization, iNOS but not 225.4 expression was suppressed, and there was no significant impact the ratio of wild-type to Δ*CPS4* mutant cells in the cecum (Figure S6).

We found that the antisymbiont IgA response not only suppresses *CPS4* expression; it also induces expression of another other capsular locus (*CPS5*; Figure 6), which, in turn, could initiate another round of host immune responses. Studies of another gut Bacteroides, *B. fragilis* (Krinos et al., 2001), have provided important insights about the complex genetic regulation of *CPS* locus expression through inversion of upstream, locus-associated promoters. We did not find any evidence of inversion in the proximal promoter of CPS4 but cannot rule out that control of CPS4 expression could be dependent on invertible elements positioned elsewhere in the *B. thetaiotaomicron* genome. Our findings provide an additional view of this regulation, one where anti-capsular antibodies modulate expression of loci producing their cognate epitopes. Gut symbionts exhibit an enormous capacity for surface variation, not only in response to immune pressure, but also as a result of other forces including nutrient availability (Sonnenburg et al., 2005; Bjursell et al., 2006). The genomes of prominent human gut Bacteroides species including *B. thetaiotaomicron*, *B. fragilis*, *B. vulgatus*, and *B. distasonis* have revealed that *CPS* loci are prominent sites of variation, in part due to lateral gene transfer (Xu et al., 2007). Moreover, comparisons of the *B. thetaiotaomicron* type strain VPI-5482 and two additional human

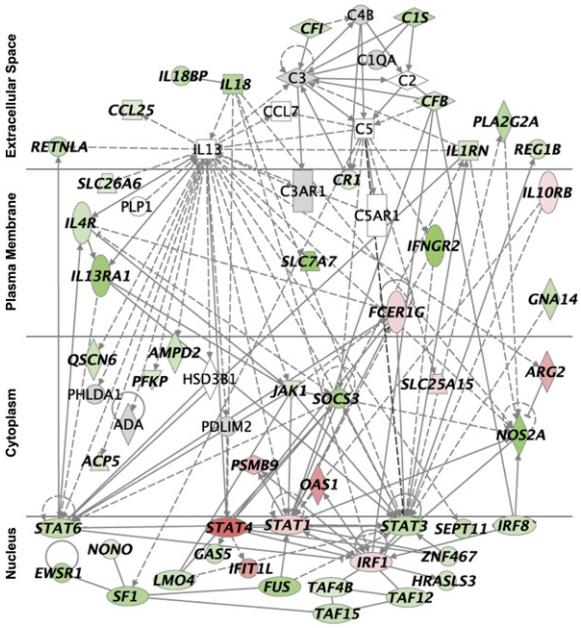


Figure 5. Ingenuity Pathway Analysis of Host Genes Differentially Expressed in *B. thetaiotaomicron*-Colonized *Rag1*$^{-/-}$ Mice without Backpacks Compared with *Rag1*$^{-/-}$ Backpack and Wild-Type B6 Animals

Mouse GeneChip analysis of the distal small intestines of animals sacrificed 10 days after colonization. In *Rag1*$^{-/-}$ mice without an antisymbiont IgA response, there is augmented induction of iNOS (*NOS2a*) and *IFNgR2* (implies the presence of IFN-γ). Complement components, IL-18 and IL-13-RA/IL-4-R, IL-1RN, PLA2G2A, and Reg1B genes are also induced. Genes downregulated in either wild-type B6 or *Rag1*$^{-/-}$ backpack animals compared to *Rag1*$^{-/-}$ mice are highlighted in green, while upregulated genes are in red. Full gene names, fold differences in expression between the different groups of mice, and p values are provided in Table S2 and Figure S5. Solid lines indicate a direct interaction between the gene products, while dashed lines indicate an indirect interaction. Genes in bold/italic were significantly changed in the comparison between *Rag1*$^{-/-}$ and *Rag1*$^{-/-}$ backpack or wild-type B6 mice. Genes highlighted by gray were called present (expressed) in the GeneChip data set, but their levels of expression were not significantly different between the groups of animals. Genes in white (open) shapes were not in the GeneChip data set but are implicated as being in the network by the Ingenuity Pathway Analysis (IPA) software.

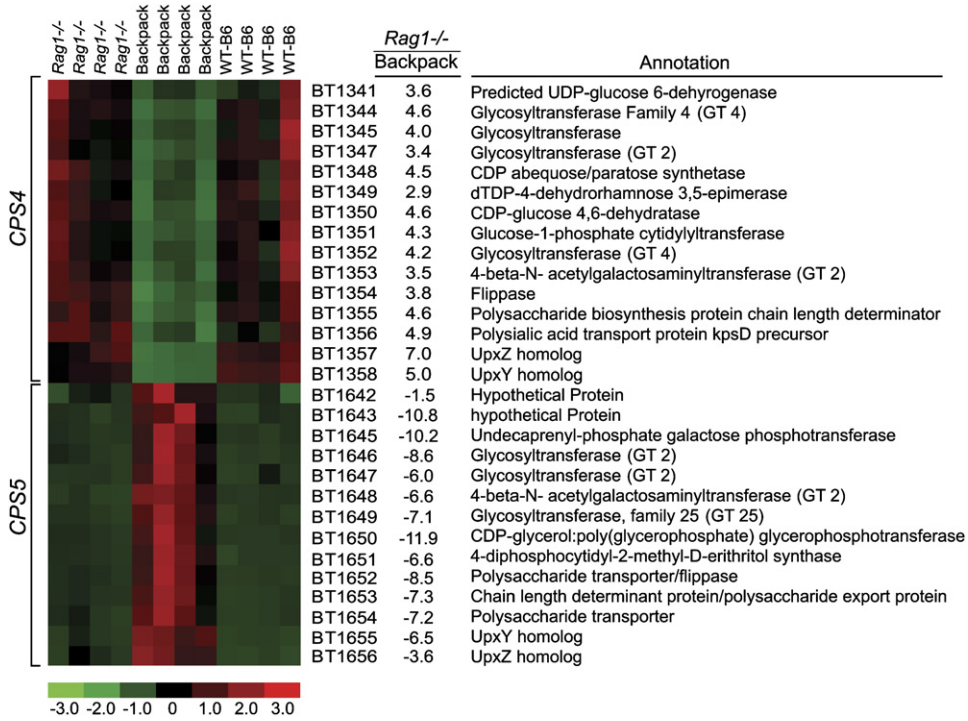
gut-derived strains we have sequenced indicate that the *CPS4* locus is highly polymorphic. Successful gut symbionts like *B. thetaiotaomicron* possess large collections of paralogous outer member proteins involved in nutrient sensing and acquisition. Thus, it seems logical to postulate that, at least from the bacterial perspective, CPS epitopes would be preferred ''bait'' for the adaptive immune system in order to minimize immune recognition of key nutrient-related proteins.

DISCUSSION

Our study supports the notion that the role for antibody in the gut is to mediate tolerance. Tolerance in the gut may be viewed as a quiescent homeostasis predicated on immune recognition of members of its microbiota. Bacteria that elicit excessive levels of IgA may suffer a greater competitive disadvantage than those that elicit low to moderate amounts. This requires that long-term residents modulate their immunodominant determinants continually, likely providing one explanation for the extraordinary level of strain-level diversity observed in the gut ecosystem. Although relatively few human gut-associated Bacteroides genomes have been sequenced, those that have reveal a large representation of genes involved in the generation of surface carbohydrates (Xu et al., 2003, 2007; Kuwahara et al., 2004; Sonnenburg et al., 2005; Cerdeno-Tarraga et al., 2005; Krinos et al., 2001; Fletcher et al., 2007). Our observations are consistent with the adaptive immune system being a driver of diversification of these surface structures, with the beneficial outcome being promotion of a noninflammatory relationship between gut symbiont and host.

The innate immune response to bacteria, including the generation of NO, is highly conserved in invertebrates and vertebrates (Davidson et al., 2004). A key evolved role of the adaptive immune system in vertebrates may be to accommodate more complex microbial communities, even at the added risk of susceptibility to colonization with pathogens, and/or autoimmunity (Hedrick, 2004; McFall-Ngai, 2007). Our results support this concept and provide evidence that a major role for the adaptive immune system is to maintain ''connection'' with the gut microbiota by selectively generating immune responses to bacteria that stimulate the innate system. This arrangement allows the host to detect new bacterial phylotypes, and to ignore the presence of those that it has previously encountered (memory). The result would be support of greater diversity without sacrificing the essential protective role of the innate immune system in maintaining the mucosal barrier.

There is an emerging model of mucosal immunity where differentiation of B cells to IgA-producing plasma cells is locally induced in the lamina propria of the small intestine by local factors (Fagarasan et al., 2001): e.g., it appears that T cell-independent class switching in the gut is induced by expression of costimulatory molecules such as CD40 ligand, APRIL, BlyS, and TGF-β by epithelial and DCs (Litinskiy et al., 2002; Macpherson and Uhr, 2004; He et al., 2007). The result of locally controlled IgA production is to create a simple feedback loop that bypasses the systemic immune system: i.e., where DCs, and epithelial cells drive IgA production independent of T cells until sufficient levels of IgA block microbial stimulation. This model could be tested in the future by creating a quasi-monoclonal (gnotobiotic) mouse with a T cell-deficient genetic background that expresses the 225.4 antibody as a B cell receptor knocked into the immunoglobulin gene locus (Cascalho et al., 1996): colonization of this mouse with *B. thetaiotaomicron* would reveal how efficiently IgA class switching occurs in a highly defined system, and the degree to which IgA production is antigen specific and T cell dependent.



	Rag1-/- / Backpack	Annotation
BT1341	3.6	Predicted UDP-glucose 6-dehyrogenase
BT1344	4.6	Glycosyltransferase Family 4 (GT 4)
BT1345	4.0	Glycosyltransferase
BT1347	3.4	Glycosyltransferase (GT 2)
BT1348	4.5	CDP abequose/paratose synthetase
BT1349	2.9	dTDP-4-dehydrorhamnose 3,5-epimerase
BT1350	4.6	CDP-glucose 4,6-dehydratase
BT1351	4.3	Glucose-1-phosphate cytidylyltransferase
BT1352	4.2	Glycosyltransferase (GT 4)
BT1353	3.5	4-beta-N- acetylgalactosaminyltransferase (GT 2)
BT1354	3.8	Flippase
BT1355	4.6	Polysaccharide biosynthesis protein chain length determinator
BT1356	4.9	Polysialic acid transport protein kpsD precursor
BT1357	7.0	UpxZ homolog
BT1358	5.0	UpxY homolog
BT1642	-1.5	Hypothetical Protein
BT1643	-10.8	hypothetical Protein
BT1645	-10.2	Undecaprenyl-phosphate galactose phosphotransferase
BT1646	-8.6	Glycosyltransferase (GT 2)
BT1647	-6.0	Glycosyltransferase (GT 2)
BT1648	-6.6	4-beta-N- acetylgalactosaminyltransferase (GT 2)
BT1649	-7.1	Glycosyltransferase, family 25 (GT 25)
BT1650	-11.9	CDP-glycerol:poly(glycerophosphate) glycerophosphotransferase
BT1651	-6.6	4-diphosphocytidyl-2-methyl-D-erithritol synthase
BT1652	-8.5	Polysaccharide transporter/flippase
BT1653	-7.3	Chain length determinant protein/polysaccharide export protein
BT1654	-7.2	Polysaccharide transporter
BT1655	-6.5	UpxY homolog
BT1656	-3.6	UpxZ homolog

Figure 6. GeneChip Analysis Reveals that Exposure to the 225.4 Antibody Downregulates *CPS4* and Upregulates *CPS5* Expression
Expression profile for *CPS4* and *CPS5* genes in the ceca of *Rag1*$^{-/-}$ mice without backpacks, in *Rag1*$^{-/-}$ backpack animals, and in wild-type B6 mice. Genes were filtered for significant differences in their expression between *Rag1*$^{-/-}$ and *Rag1*$^{-/-}$ backpack animals as defined by >1.5-fold difference and a p value < 0.05 (determined by Student's t test in dChip).

One extrapolation from our observations is that inflammatory bowel disease (IBD) could be viewed, at least in part, as a failure to generate an effective adaptive immune response to the resident gut microbiota: such a response would normally prevent presentation of microbial antigens to T cells specific for members of the community. Consistent with this model, the antibody response of *TCR*$\alpha^{-/-}$ mice to gut microbes changes from polyclonal to oligoclonal as they develop colitis. Moreover, passive transfer of Ig into this model ameliorates disease (Mizoguchi et al., 1996, 1997).

Thus, instead of just searching for microbial targets that are enriched in the IBD-associated antibody repertoire, identifying where "holes" exist in the repertoire may be informative and could suggest new therapeutic strategies based on active or passive immunization. The identification of such holes should be facilitated by the upcoming human microbiome project, which will sequence (1) the genomes of a large number of cultured representatives of the phylogenetic types present in the gut microbiota, and (2) samples of gut microbial communities obtained from healthy individuals as well as those with diseases such as IBD. Moreover, these concepts likely apply to other human body habitats that harbor a microbiota and can be further explored using elaborations of the type of gnotobiotic mouse model described in this report.

EXPERIMENTAL PROCEDURES

Colonization of Germ-Free Mice

C57Bl/6J (B6) mice were obtained from the Jackson Laboratory. Germ-free B6 wild-type and *Rag1*$^{-/-}$ mice were housed in gnotobiotic isolators (Hooper et al., 2002) under a strict 12 hr light cycle and fed an autoclaved, polysaccharide-rich, standard rodent chow diet (B&K Universal, East Yorkshire, UK). All experiments were performed using protocols approved by the Washington University Animal Studies Committee.

Six- to eight-week-old males were colonized with a single gavage of 10^8 CFU of wild-type *B. thetaiotaomicron* strain VPI-5482, and/or an isogenic mutant strain with a disrupted *CPS4* locus (see below). Bacteria were harvested from stationary phase cultures in TYG medium (1% tryptone, 0.5% yeast extract, 0.2% glucose) supplemented with 100 mM potassium phosphate buffer (pH 7.2), 4.1 mM cysteine, 200 mM histidine, 6.8 mM $CaCl_2$, 140 nM $FeSO_4$, 81 mM $MgSO_4$, 4.8 mM $NaHCO_3$, 1.4 mM NaCl, 1.9 mM hematin, and 5.8 mM vitamin K_3.

Hybridomas Obtained from Lamina Propria Fusions

Gnotobiotic mice were killed 2 weeks after gavage with wild-type *B. thetaiotaomicron*. The small intestine from a given mouse was immediately removed and flushed with Dulbecco's modified Eagle's medium (DMEM) containing penicillin (100 units/ml) and streptomycin (100 μg/ml). The gut was then opened along its cephalocaudal axis and minced into small (~2–5 mm) pieces. Following a 15 min incubation at 37°C in 50 ml Hank's buffered salt solution (HBSS) containing 5 mM EDTA, tissue fragments were placed in DMEM containing collagenase (100 units/ml; Sigma, St. Louis, MO) and dispase (0.5 units/ml; Fisher) and incubated for 2 hr at 37°C, with vigorous shaking for 30 s every 30 min. Released cells were separated from the remaining tissue

fragments by sedimentation on the bench-top for 1 min, and the resulting cell suspension (supernatant fraction) was filtered through a Nytex filter (70 μm diameter pore; BD Biosciences, Bedford, MA). Cells in the filtrate were then combined with the myeloma fusion partner (P3X63.Ag8); the combined population was washed three times in serum-free DMEM medium, and the cells were fused by adding PEG1500 (50% w/v; Roche, Mannheim, Germany; Kohler and Milstein, 1975). The ratio of IgA-, IgM-, and IgG2b-producing hybridomas was 7:11:14 (n = 61 colonies scored representing three animals; note that control experiments using intestinal lamina propria lymphocytes recovered from age-matched, conventionally raised wild-type B6 animals produced hybridomas with a similar isotype distribution).

B. thetaiotaomicron ELISAs

All ELISAs were performed using standard protocols (Velazquez et al., 2001) in 96-well plates (Nunc-Maxisorb, Nalge-Nunc, Rochester, NY). Bacterial strains suspended in PBS, lysates prepared from strains, capsular polysaccharides purified from strains, or extracts of cecal contents harvested from colonized gnotobiotic mice were assayed by ELISA (see following paragraphs for descriptions of how samples were prepared).

Samples to be assayed were diluted in sodium bicarbonate coating buffer (15 mM sodium carbonate, 35 mM sodium bicarbonate, and 3 mM sodium azide [pH 8.5]). All steps were performed either at 4°C overnight, or room temperature for 2 hr. Following addition of supernatants from hybridomas (~1–5 μg/IgA/well), goat horseradish peroxidase (HRP)-conjugated anti-mouse IgA (Southern Biotech; 1:1000 dilution, 50 μl/well) was added. All ELISAs were developed using ABTS [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] (1 mM; Roche) in citrate buffer (100 mM citric acid, 50 mM sodium phosphate [pH 4.2]) containing 0.03% H_2O_2. Reactions were read in an ELISA plate reader (ThermoMax, Molecular Devices) at O.D. 405 nm.

Bacterial Lysates

Lysates were generated by suspending 10^{10} CFU of bacteria in 0.5 ml of sterile PBS and sonicating the mixture in a Misonix XL-2020 sonicator (10 min at a setting of "10"). This mixture was then diluted 1:1000 in bicarbonate buffer, and a 50 μl aliquot was added to each well of an ELISA plate. Lysates contained capsular antigens, membrane and cell wall antigens, plus intracellular antigens and were therefore used for initial screening of hybridoma fusions.

Capsular Polysaccharides

Capsular polysaccharides were isolated from *B. thetaiotaomicron* VPI-5482 cells, harvested at the stationary phase of growth in TYG medium, using the hot water-phenol method (Jann et al., 1965). Briefly, bacteria were extracted for 2 hr in phenol:water (1:1) at 65°C with constant stirring. Phases were separated by gravity, and the top phase was dialyzed for 3 days at 4°C against tap water. The dialyzed material was subsequently lyophilized and suspended in sterile PBS (1 mg/ml). LPS was removed by ultracentrifugation (65,000 × g for 2 hr at 4°C).

Cecal Contents

Extractions were carried at 65°C in 1.5 ml eppendorf tubes containing 0.5 ml of a 1:100 dilution of cecal contents in PBS, and 0.5 ml phenol. Samples were vortexed for 30 s, every 30 min over a 2 hr period. Following centrifugation at 20,000 × g for 30 min at 4°C, the upper phase was recovered, serial dilutions (in bicarbonate buffer) were made, and ELISA was performed. To estimate the amount of 225.4 epitope present in cecal contents, ELISA reactivity was standardized to a curve constructed from serial dilutions of *B. thetaiotaomicron* (grown in TYG to stationary phase) into bicarbonate buffer. This stationary culture was arbitrarily designated as having 1×10^9 units per ml (the equivalent of CFU under these conditions). Therefore, in TYG-grown *B. thetaiotaomicron*, one 225.4 epitope unit is the equivalent to one colony-forming unit.

Isotype ELISA

ELISA plates were coated overnight at 4°C with goat anti-mouse Ig (Southern Biotech; reacts with heavy and light chains), then washed and blocked with 1% BSA-PBS (30 min at room temperature). Serial dilutions of serum samples, obtained from *B. thetaiotaomicron*-colonized mice at the time of their sacrifice, were added to the 96-well plates (n = 3 biological replicates/per time point), followed by HRP-conjugated, isotype-specific, secondary antibodies (1:1000 dilution in PBS-BSA; Southern Biotech). Fold change at each time point following colonization was calculated as the isotype level of each mouse relative to the mean of the germ-free control group.

Transposon Mutagenesis

Mutagenesis was performed using the donor plasmid pEP4351 and *B. thetaiotaomicron* VPI-5482 (Salyers et al., 2000; Shoemaker et al., 1986). Mutants were isolated based on their growth on Brain Heart Infusion-sheep red blood cell agar plates containing erythromycin (10 μg ml; to select for transposon-containing *B. thetaiotaomicron*) and 600 μg/ml gentamicin (to select against any persistent *E. coli*). Individual colonies were picked into 96-well plates and grown overnight in TYG medium in anaerobic jars. Plates were split: one replicate was frozen in 25% glycerol for later studies; the other plate was frozen for subsequent ELISA screening with the 225.4 mAb.

Sites of transposon insertion in 225.4 antibody-negative colonies were identified using an arbitrarily primed polymerase chain reaction (AP-PCR). The AP-PCR protocol consists of a nested PCR with the following primers: Round 1, S3794 (5′-ATCAGTATGCTTTGTGTGTG) and either AR7 [5′-GGCCACGCGTCGACTAGTAC(N)$_{10}$GTAAT] or AR8 [5′-GGCCACGCGTCGACTAGTAC(N)$_{10}$GATGC]; Round 2, ISF (5′-TCG GTTATATGTTTGCTCATCTGC) and AR2 (5′-GGCCACGCGTCGACTA GTAC) (7). The product of the second round of PCR was purified (QIAquick PCR Purification Kit; QIAGEN) and then sequenced from the right arm of the transposon into the chromosomal DNA using primer IS4908S (5′-ATCCATTCAGAGTGAGAGAAAG). The results were compared to the genome sequence of *B. thetaiotaomicron* VPI-5481 (GenBank accession number NC_004663) and Tn4351 (accession number M17124): sequences aligning to both were considered to be a positive hit.

pGERM-Directed Mutagenesis of *CPS* Loci

The first gene in every *CPS* locus was targeted using the pGERM suicide vector (Xu et al., 2003; Sonnenburg et al., 2005). Mutagenesis was performed using protocols described by Hooper et al. (1999). The site of insertion of pGERM was verified by PCR (see Table S5 for a list of primers), and by sequencing.

Backpack Experiments

Hybridomas were grown to 50%–75% confluency in DMEM and washed three times in pyrogen-free saline. Cells were introduced into the gnotobiotic isolators in a way that preserved sterility (see Crawford and Gordon, 2005 for details) and then injected subcutaneously into 6-to 8-week-old male B6 *Rag1*$^{-/-}$ recipients (2×10^6 cells/mouse; n = 16–20 mice/experiment; three independent experiments). Ten days after implantation, each mouse was colonized with a single gavage of 10^8 CFU of *B. thetaiotaomicron* (with or without the Δ*CPS4* isogenic mutant). Mice were then sacrificed 10 days following colonization, and cecal contents were recovered. The small intestine was rapidly divided into 16 equal length segments, and segments 9–12 and 14 were snap frozen in liquid nitrogen for subsequent isolation of total cellular RNA (see below).

The Impact of Anti-*B. thetaiotaomicron* on Bacterial RNA Expression In Vivo

Cecal contents were flash frozen in liquid nitrogen immediately after their harvest from each mouse in each treatment group (n = 4 mice/group; selected based on cecal IgA levels contents [range of cecal IgA, 9–153 μg/ml; geometric mean, 38 μg/ml]) and stored at −80°C until use. An aliquot (~200 mg) was thawed in 2–3 volumes of

RNAProtect (QIAGEN) and centrifuged (3000 × g for 10 min), and 500 μl of 200 mM NaCl/20 mM EDTA was added to the resulting pellet, together with 200 μl of 20% SDS and 500 μl phenol:chloroform:isoamyl alcohol (125:24:1; pH 4.5; Ambion). Acid-washed silica beads (Sigma; 212–300 μm diameter; 250 mg) were introduced, and bacteria were lysed (Mini-Beadbeater; Biospec; "high" setting for 5 min at room temperature). Following centrifugation (13,000 × g for 3 min at 4°C), the sample was re-extracted in phenol:chloroform:isoamyl alcohol, precipitated with 60 μl of 3 M sodium acetate (pH 5.2) plus 600 μl cold isopropanol, and RNA was purified (RNEasy kit; QIAGEN; note that residual genomic DNA was subsequently removed by treatment with DNAfree; Ambion). The relative proportion of bacterial versus host RNA in each preparation was defined by qRT-PCR, using primers directed at *B. thetaiotaomicron* 16S rRNA (forward, 5′-GGTAGTCCACA CAGTAAACGATGAA; reverse, 5′-CCCGTCAATTCCTTTGAGTTTC) and mouse 18S rRNA (forward, 5′-CATTCGAACGTCTGCCCTATC; reverse, 5′-CCTGTGCCTTCCTTGGA). The results revealed that ≥90% of the recovered rRNA in each preparation was bacterial. GeneChip targets were prepared as described (Sonnenburg et al., 2005). The cDNA product was isolated (QiaQuick Spin columns; QIAGEN), fragmented (DNase-I; Amersham Biosciences), and biotinylated (Enzo-BioArray Terminal Labeling Kit). Standard Affymetrix protocols were used for hybridization of the cDNA targets to each *B. thetaiotaomicron* GeneChip (Sonnenburg et al., 2005, 2006).

GeneChip data were analyzed as follows. After normalization, model-based expression values were generated (PM-MM model). Signals from spike-in control transcripts and oligo-B2 (Affymetrix) were used to assess the quality of the target preparation and target hybridization, respectively. Comparisons were performed on specified GeneChip data sets to identify genes up- or downregulated in the experimental group (E) relative to the baseline group (B). Criteria used to determine significant changes in expression were guided by the median false discovery rate of 50 permutations in which samples were randomly shuffled between groups. The following criteria were routinely used to identify significantly upregulated genes while maintaining an empirical false discovery rate of less than 0.05: (1) E/B > 1.5; (2) E-B ≥ 100; (3) E = B $p \leq 0.05$; and (4) called "present" in ≥75% of "E" GeneChips.

Profiling Intestinal Gene Expression

RNA prepared from small intestinal segments 9–12 (treated as a single domain) and segment 14 from each mouse in each treatment group were combined proportionally (4:1) for each mouse. Targets were then prepared and hybridized to Affymetrix Mouse Genome Moe430_2 GeneChips using standard protocols (Affymetrix). GeneChip data sets were analyzed using DNA-chip analyzer (dChip). Genes up- or downregulated in *Rag1*$^{-/-}$ mice with backpack hybridomas or wild-type B6 animals (E) relative to *Rag1*$^{-/-}$ mice without backpacks (B) were identified using the following criteria: (1) E/B > 1.2; (2) called "present" in at least two of three "E" GeneChips; and (3) E = B $p < 0.05$.

Real-Time Quantitative RT-PCR

Aliquots (2 ng) of the intestinal RNAs described above were reverse transcribed in 20 μl reactions containing Superscript II RT (200 units; Invitrogen) and oligo-dT_{15} (50 ng/μl; Roche). An aliquot (2 μl) containing the cDNA product was then added to 25 μl qRT-PCR reaction mixtures together with Sybr-green reagent (12.5 μl; ABgene, Rochester, NY) and primers specific for iNOS (5′-CAGCTGGGCTGTACAAACC TT; 5′-CATTGGAAGTGAAGCGTTTCG; final concentration, 900 nM). Assays were performed in triplicate using a Mx3000P QPCR System instrument (Stratagene). Transcript levels within samples were normalized to ribosomal protein L32 mRNA (5′-CCTCTGGTGAAGCCCAA GATC; 5′-TCTGGGTTTCCGCCAGTTT), and fold changes were determined for each colonized mouse referenced against the lowest value within the set (a wild-type B6 mouse) ($\Delta\Delta C_T$ method) (Sonnenburg et al., 2006; Stappenbeck et al., 2002).

RNA prepared from cecal contents was also subjected to qRT-PCR analysis of *BT1348* gene expression. In this case, the reverse transcription reaction mix contained 75 ng of random hexamer primers (Invitrogen). Transcript levels were normalized to 16S rRNA expression (see Table S5 for primers).

GeneChip data were submitted to Gene Expression Omnibus (GEO) under accession numbers GSE9018 and GSE9019.

Supplemental Data

The Supplemental Data include six supplemental figures and five supplemental tables and can be found with this article online at http://www.cellhostandmicrobe.com/cgi/content/full/2/5/328/DC1/.

ACKNOWLEDGMENTS

We thank David O'Donnell and Maria Karlsson for invaluable help with husbandry of gnotobiotic mice, Sabrina Wagoner for expert technical assistance, Jennifer Gill and Abigail Salyers for contributions to transposon mutagenesis of *B. thetaiotaomicron*, and Emil Unanue and members of the Gordon lab for many helpful discussions. This work was supported by grants from the NIH (DK30292, T32HD07409, T32CA09547).

Received: June 19, 2007
Revised: August 17, 2007
Accepted: September 19, 2007
Published: November 14, 2007

REFERENCES

Bjursell, M.K., Martens, E.C., and Gordon, J.I. (2006). Functional genomic and metabolic studies of the adaptations of a prominent adult human gut symbiont, Bacteroides thetaiotaomicron, to the suckling period. J. Biol. Chem. *281*, 36269–36279.

Cascalho, M., Ma, A., Lee, S., Masat, L., and Wabl, M. (1996). A quasi-monoclonal mouse. Science *272*, 1649–1652.

Cash, H.L., Whitham, C.V., Behrendt, C.L., and Hooper, L.V. (2006). Symbiotic bacteria direct expression of an intestinal bactericidal lectin. Science *313*, 1126–1130.

Cerdeno-Tarraga, A.M., Patrick, S., Crossman, L.C., Blakely, G., Abratt, V., Lennard, N., Poxton, I., Duerden, B., Harris, B., Quail, M.A., et al. (2005). Extensive DNA inversions in the B. fragilis genome control variable gene expression. Science *307*, 1463–1465.

Cobb, B.S., Hertweck, A., Smith, J., O'Connor, E., Graf, D., Cook, T., Smale, S.T., Sakaguchi, S., Livesey, F.J., Fisher, A.G., and Merkenschlager, M. (2006). A role for Dicer in immune regulation. J. Exp. Med. *203*, 2519–2527.

Coughlin, R.T., White, A.C., Anderson, C.A., Carlone, G.M., Klein, D.L., and Treanor, J. (1998). Characterization of pneumococcal specific antibodies in healthy unvaccinated adults. Vaccine *16*, 1761–1767.

Crawford, P.A., and Gordon, J.I. (2005). Microbial regulation of intestinal radiosensitivity. Proc. Natl. Acad. Sci. USA *102*, 13254–13259.

Davidson, S.K., Koropatnick, T.A., Kossmehl, R., Sycuro, L., and McFall-Ngai, M.J. (2004). NO means 'yes' in the squid-vibrio symbiosis: Nitric oxide (NO) during the initial stages of a beneficial association. Cell. Microbiol. *6*, 1139–1151.

Fagarasan, S., Kinoshita, K., Muramatsu, M., Ikuta, K., and Honjo, T. (2001). In situ class switching and differentiation to IgA-producing cells in the gut lamina propria. Nature *413*, 639–643.

Fagarasan, S., Muramatsu, M., Suzuki, K., Nagaoka, H., Hiai, H., and Honjo, T. (2002). Critical roles of activation-induced cytidine deaminase in the homeostasis of gut flora. Science *298*, 1424–1427.

Fletcher, C.M., Coyne, M.J., Bentley, D.L., Villa, O.F., and Comstock, L.E. (2007). Phase-variable expression of a family of glycoproteins

imparts a dynamic surface to a symbiont in its human intestinal ecosystem. Proc. Natl. Acad. Sci. USA *104*, 2413–2418.

Frank, D.N., St. Amand, A.L., Feldman, R.A., Boedeker, E.C., Harpaz, N., and Pace, N.R. (2007). Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc. Natl. Acad. Sci. USA *104*, 13780–13785.

Gerth, A.J., Lin, L., Neurath, M.F., Glimcher, L.H., and Peng, S.L. (2004). An innate cell-mediated, murine ulcerative colitis-like syndrome in the absence of nuclear factor of activated T cells. Gastroenterology *126*, 1115–1121.

He, B., Xu, W., Santini, P.A., Polydorides, A.D., Chiu, A., Estrella, J., Shan, M., Chadburn, A., Villanacci, V., Plebani, A., et al. (2007). Intestinal bacteria trigger T cell-independent immunoglobulin A(2) class switching by inducing epithelial-cell secretion of the cytokine APRIL. Immunity *26*, 812–826.

Hedrick, S.M. (2004). The acquired immune system: A vantage from beneath. Immunity *21*, 607–615.

Hooper, L.V., Xu, J., Falk, P.G., Midtvedt, T., and Gordon, J.I. (1999). A molecular sensor that allows a gut commensal to control its nutrient foundation in a competitive ecosystem. Proc. Natl. Acad. Sci. USA *96*, 9833–9838.

Hooper, L.V., Mills, J.C., Roth, K.R., Stappenbeck, T.S., Wong, M.H., and Gordon, J.I. (2002). Combining gnotobiotic mouse models with functional genomics to define the impact of the microflora on host physiology. Molecular Cellular Microbiol. *31*, 559.

Izcue, A., Coombes, J.L., and Powrie, F. (2006). Regulatory T cells suppress systemic and mucosal immune activation to control intestinal inflammation. Immunol. Rev. *212*, 256–271.

Jann, K., Jann, B., Orskov, F., Orskov, I., and Westphal, O. (1965). Immunochemical studies of K antigens from Escherichia coli. II. K antigen from E. coli 08:K42(A):H-. Biochem. Z. *342*, 1–22.

Kanai, T., Kawamura, T., Dohi, T., Makita, S., Nemoto, Y., Totsuka, T., and Watanabe, M. (2006). TH1/TH2-mediated colitis induced by adoptive transfer of CD4+CD45RBhigh T lymphocytes into nude mice. Inflamm. Bowel Dis. *12*, 89–99.

Keilbaugh, S.A., Shin, M.E., Banchereau, R.F., McVay, L.D., Boyko, N., Artis, D., Cebra, J.J., and Wu, G.D. (2005). Activation of RegIIIbeta/gamma and interferon gamma expression in the intestinal tract of SCID mice: An innate response to bacterial colonisation of the gut. Gut *54*, 623–629.

Kohler, G., and Milstein, C. (1975). Continuous cultures of fused cells secreting antibody of predefined specificity. Nature *256*, 495–497.

Krinos, C.M., Coyne, M.J., Weinacht, K.G., Tzianabos, A.O., Kasper, D.L., and Comstock, L.E. (2001). Extensive surface diversity of a commensal microorganism by multiple DNA inversions. Nature *414*, 555–558.

Kuwahara, T., Yamashita, A., Hirakawa, H., Nakayama, H., Toh, H., Okada, N., Kuhara, S., Hattori, M., Hayashi, T., and Ohnishi, Y. (2004). Genomic analysis of Bacteroides fragilis reveals extensive DNA inversions regulating cell surface adaptation. Proc. Natl. Acad. Sci. USA *101*, 14919–14924.

Landers, C.J., Cohavy, O., Misra, R., Yang, H., Lin, Y.C., Braun, J., and Targan, S.R. (2002). Selected loss of tolerance evidenced by Crohn's disease-associated immune responses to auto- and microbial antigens. Gastroenterology *123*, 689–699.

Ley, R.E., Peterson, D.A., and Gordon, J.I. (2006a). Ecological and evolutionary forces shaping microbial diversity in the human intestine. Cell *124*, 837–848.

Ley, R.E., Turnbaugh, P.J., Klein, S., and Gordon, J.I. (2006b). Microbial ecology: Human gut microbes associated with obesity. Nature *444*, 1022–1023.

Litinskiy, M.B., Nardelli, B., Hilbert, D.M., He, B., Schaffer, A., Casali, P., and Cerutti, A. (2002). DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. Nat. Immunol. *3*, 822–829.

Macpherson, A.J., Gatto, D., Sainsbury, E., Harriman, G.R., Hengartner, H., and Zinkernagel, R.M. (2000). A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. Science *288*, 2222–2226.

Macpherson, A.J., and Uhr, T. (2004). Induction of protective IgA by intestinal dendritic cells carrying commensal bacteria. Science *303*, 1662–1665.

Mazmanian, S.K., Liu, C.H., Tzianabos, A.O., and Kasper, D.L. (2005). An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell *122*, 107–118.

McFall-Ngai, M. (2007). Adaptive immunity: Care for the community. Nature *445*, 153.

Michetti, P., Mahan, M.J., Slauch, J.M., Mekalanos, J.J., and Neutra, M.R. (1992). Monoclonal secretory immunoglobulin A protects mice against oral challenge with the invasive pathogen Salmonella typhimurium. Infect. Immun. *60*, 1786–1792.

Mizoguchi, A., Mizoguchi, E., Tonoegawa, S., and Bhan, A.K. (1996). Alteration of a polyclonal to an oligoclonal immune response to cecal aerobic bacteril antigens in TCR alpha mutant mice with inflammatory bowel disease. Int. Immunol. *8*, 1387–1394.

Mizoguchi, A., Mizoguchi, E., Smith, R.N., Preffer, F.I., and Bhan, A.K. (1997). Suppressive role of B cells in chronic colitis of T cell receptor alpha mutant mice. J. Exp. Med. *186*, 1749–1756.

Rodionov, D.A., Dubchak, I.L., Arkin, A.P., Alm, E.J., and Gelfand, M.S. (2005). Dissimilatory metabolism of nitrogen oxides in bacteria: Comparative reconstruction of transcriptional networks. PLoS Comput. Biol. *1*, e55.

Salyers, A.A., Bonheyo, G., and Shoemaker, N.B. (2000). Starting a new genetic system: Lessons from bacteroides. Methods *20*, 35–46.

Shoemaker, N.B., Getty, C., Gardner, J.F., and Salyers, A.A. (1986). Tn4351 transposes in Bacteroides spp. and mediates the integration of plasmid R751 into the Bacteroides chromosome. J. Bacteriol. *165*, 929–936.

Shroff, K.E., Meslin, K., and Cebra, J.J. (1995). Commensal enteric bacteria engender a self-limiting humoral mucosal immune response while permanently colonizing the gut. Infect. Immun. *63*, 3904–3913.

Sonnenburg, J.L., Xu, J., Leip, D.D., Chen, C.H., Westover, B.P., Weatherford, J., Buhler, J.D., and Gordon, J.I. (2005). Glycan foraging in vivo by an intestine-adapted bacterial symbiont. Science *307*, 1955–1959.

Sonnenburg, J.L., Chen, C.T., and Gordon, J.I. (2006). Genomic and metabolic studies of the impact of probiotics on a model gut symbiont and host. PLoS Biol. *4*, e413.

Stappenbeck, T.S., Hooper, L.V., Manchester, J.K., Wong, M.H., and Gordon, J.I. (2002). Laser capture microdissection of mouse intestine: Characterizing mRNA and protein expression, and profiling intermediary metabolism in specified cell populations. Methods Enzymol. *356*, 167–196.

Suzuki, K., Meek, B., Doi, Y., Muramatsu, M., Chiba, T., Honjo, T., and Fagarasan, S. (2004). Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. Proc. Natl. Acad. Sci. USA *101*, 1981–1986.

Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R., and Gordon, J.I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. Nature *444*, 1027–1031.

Velazquez, C., DiPaolo, R., and Unanue, E.R. (2001). Quantitation of lysozyme peptides bound to class II MHC molecules indicates very large differences in levels of presentation. J. Immunol. *166*, 5488–5494.

Westover, B.P., Buhler, J.D., Sonnenburg, J.L., and Gordon, J.I. (2005). Operon prediction without a training set. Bioinformatics *21*, 880–888.

Xu, J., Bjursell, M.K., Himrod, J., Deng, S., Carmichael, L.K., Chiang, H.C., Hooper, L.V., and Gordon, J.I. (2003). A genomic view of the human-Bacteroides thetaiotaomicron symbiosis. Science *299*, 2074–2076.

Xu, J., Mahowald, M.A., Ley, R.E., Lozupone, C.A., Hamady, M., Martens, E.C., Henrissat, B., Coutinho, P.M., Minx, P., Latreille, P., et al. (2007). Evolution of symbiotic bacteria in the distal human intestine. PLoS Biol. *5*, e156.

Accession Numbers

GeneChip data sets are available in the Gene Expression Omnibus repository under the accession numbers GSE9018, GSE9019, and GSE6504.