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Host-Mediated Inflammation Disrupts the Intestinal Microbiota and Promotes the Overgrowth of Enterobacteriaceae

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SUMMARY

While the normal microbiota has been implicated as a critical defense against invading pathogens, the impact of enteropathogenic infection and host inflammation on intestinal microbial communities has not been elucidated. Using mouse models of *Citrobacter rodentium*, which closely mimics human diarrheal pathogens inducing host intestinal inflammation, and *Campylobacter jejuni* infection, as well as chemically and genetically induced models of intestinal inflammation, we demonstrate that host-mediated inflammation in response to an infecting agent, a chemical trigger, or genetic predisposition markedly alters the colonic microbial community. While eliminating a subset of indigenous microbiota, host-mediated inflammation supported the growth of either the resident or introduced aerobic bacteria, particularly of the Enterobacteriaceae family. Further, assault by an enteropathogen and host-mediated inflammation combined to significantly reduce the total numbers of resident colonic bacteria. These findings underscore the importance of intestinal microbial ecosystems in infectious colitis and noninfectious intestinal inflammatory conditions, such as inflammatory bowel disease.

INTRODUCTION

Animals are colonized by a large number of commensal and symbiotic microorganisms, collectively referred to as the normal microbiota. The intestinal system is the most heavily colonized body site, with concentrations ranging between 10^3 cells per milliliter in the stomach up to 10^{11} bacterial cells per gram in the colon (Xu and Gordon, 2003). The intestinal bacterial community is composed of at least 500 different bacterial species, most of which are as yet unculturable (Eckburg et al., 2005). Obligate anaerobes make up most of the colonic microbiota, with facultative anaerobes occurring in ~1000-fold lower numbers (Gibson and Roberfroid, 1995) and the phyla Firmicutes and Bacteroidetes dominating both the human and the murine intestinal microbiota (Eckburg et al., 2005; Ley et al., 2005). Proteobacteria, Actinobacteria, Verrucomicrobia, and Cyanobacteria phyla have also been detected in humans and mice and Fusobacteria in humans only. Within each phylotype, high numbers of different species are found which show numerous interindividual variations.

Bacterial colonization of the intestine is essential for many normal physiological processes, such as building the intestinal epithelial barrier, enhancing intestinal development and nutrient acquisition, development of the immune system, and in the defense against pathogens (Hooper et al., 2002; Sansonetti, 2004). The contribution of the microbiota to pathogenic colonization resistance may be accomplished through a multitude of factors. For example, pathogenic bacteria must compete with the resident microbiota for space, nutrients, and host receptors (Hooper et al., 2002; Reid et al., 2001; Sprunt and Leidy, 1988), and the intestinal microbiota isolates and microbial metabolism by-products have been shown to exert an inhibitory effect on a number of Gram-negative pathogenic microorganisms (Alakomi et al., 2000; Silva et al., 2001; Servin, 2004; Gomes et al., 2006).

The intestinal microbiota is also crucially important for proper development of the gut-associated lymphoid tissue, or GALT (Shi and Walker, 2004), increasing the number of active intraepithelial lymphocytes, as well as shifting the α,β- to γ,δ-TCR balance (Imaoka et al., 1996). Different *Lactobacillus* species, an important member of gut microbiota, differentially activate dendritic cells, inducing them to produce different arrays of inflammatory cytokines, and thus playing a major role in the modulation of the Th1, Th2 and Th3 balance (Christensen et al., 2002). Recently, PSA of *Bacteroides fragilis*, a member of the microbiota, was implicated as the archetypal molecule that mediates the maturation of the host intestinal immune system (Mazmanian et al., 2005). The establishment of a resident population of immunocytes keeps the intestinal epithelium in a state of "physiological inflammation," which is required for the generation of a rapid defense response (Sansonetti, 2004).

While some of the factors by which the microbiota prevents infection have been elucidated, our knowledge of

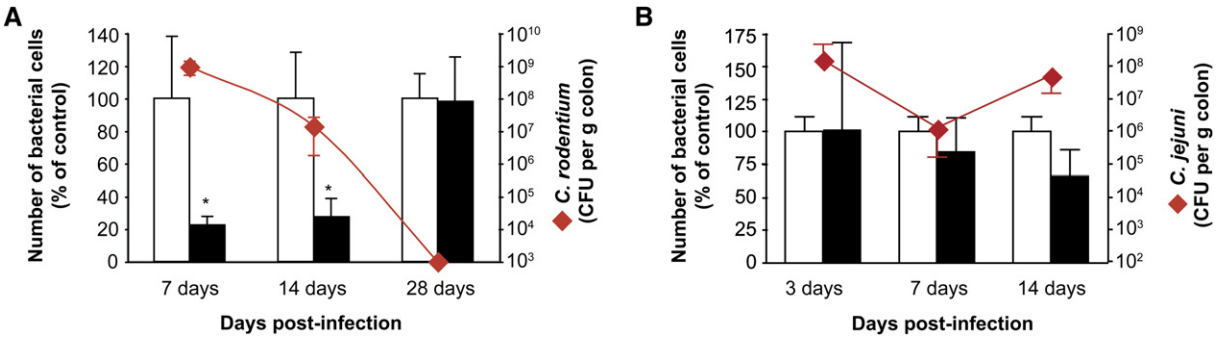

Figure 1. *C. rodentium*, but Not *C. jejuni* Infection, Results in a Significant Reduction of Total Intestinal Microbes

The total number of bacteria was determined by SYBR Green DNA staining in infected (black bars) and control (white bars) animals. Red diamonds represent *C. rodentium* cfu on Mac Conkey agar or *C. jejuni* cfu on Mueller Hinton agar.

(A) *C. rodentium* infection groups. n = 6 mice per group, error bars represent SD; *p < 0.005 two-tailed t test; each time point was assessed in three different experiments with the same outcome.

(B) *C. jejuni* infection groups. n = 4 mice for 3 days infected, n = 5 mice for other groups, error bars represent SD, no significant differences were observed between the different groups; 3 days time point was assessed once, 7 days time point was assessed three times, and 14 days time point was assessed twice with the same outcome.

the response of the intestinal microbial community to infection is extremely limited (Kuehl et al., 2005; Aebischer et al., 2006; Whary et al., 2006). Enteropathogenic infection and the infection-induced intestinal inflammation effect dramatic changes in the intestinal ecosystem, suggesting a hypothesis that changes in the composition and/or total numbers of the intestinal microbiota will occur as a result. The prospective perturbations in the intestinal microbiota could be dependent on the infecting bacterium, the host response to it, or a combination of both factors. In order to investigate these potential changes in the microbiota, we used both *Citrobacter rodentium*, a close relative of the human diarrheal pathogens enterohemorrhagic and enteropathogenic *Escherichia coli* (EHEC and EPEC), as a model of pathogen-induced intestinal inflammation (Luperchio and Schauer, 2001), and *Campylobacter jejuni*, which colonizes the mouse intestinal tract but fails to induce an inflammatory response (Mansfield et al., 2007; Rinella et al., 2006), to assess the effect of colonization in the absence of inflammation upon the host microbiota. In addition, we used a dextran sodium sulfate (DSS)-induced intestinal inflammation model (Wirtz et al., 2007) and IL-10 KO mice genetically predisposed to inflammation (Shull et al., 1992; Kuhn et al., 1993) to investigate the role of inflammation alone on microbiota changes. We found that host-mediated inflammation alone is sufficient to perturb the composition of the intestinal microbiota, eliminating a subset of bacteria while supporting the growth of others, whereas both enteropathogenic infection and host inflammatory response are necessary to significantly reduce the total numbers of microbiota.

RESULTS

Pathogen-Induced Inflammation Reduces the Numbers of the Mammalian Intestinal Microbiota

Using SYBR green DNA staining we determined that colons of healthy uninfected C57BL/6 mice contained approximately 10^{10} bacteria/g colon, consistent with previously reported numbers (Xu and Gordon, 2003). Next, we wanted to investigate the changes that occur in the levels of colonic microbial population upon infection with *C. rodentium* and *C. jejuni*.

C. rodentium is a well described murine pathogen that closely resembles human diarrheal pathogens EPEC and EHEC (Luperchio and Schauer, 2001). It belongs to the Enterobacteriaceae family of the γ-Proteobacteria class in the phylum Proteobacteria. This family contains additional important pathogens, such as *Salmonella*, *Shigella*, and *Yersinia*. Infection with *C. rodentium* is well documented to cause colonic hyperplasia and inflammation (Luperchio and Schauer, 2001).

We determined the number of microbial cells in mouse colons at different stages of *C. rodentium* infection: when *C. rodentium* intestinal counts are maximal (7 days postinfection [dpi]), at the beginning of *C. rodentium* clearance (14 dpi), and after infection is resolved (28 dpi). *C. rodentium*-induced intestinal inflammation is manifest starting 7 dpi, becomes maximal at 2 to 3 weeks, and is resolved with resolution of infection, i.e., by 28 dpi (Luperchio and Schauer, 2001). Infected mouse colons were colonized by significantly fewer microbial cells than those of uninfected mice; this reduction was approximately 4-fold at 7 and 14 dpi, as determined by SYBR green DNA staining, and coincided with high levels of *C. rodentium* colonization and intestinal pathology (Figure 1A). Interestingly, after resolution of the infection, total bacterial counts increased and returned to levels indistinguishable from uninfected control animals (Figure 1A).

C. jejuni is an important cause of diarrheal outbreaks with potential neurological complications in both the developing and the developed world (Coker et al., 2002); however, it is not a natural murine pathogen. Although it is able to colonize the murine intestine, pathology does not develop in immunocompetent hosts (Rinella et al., 2006; Mansfield et al., 2007; O.L.C., E.C.G., and B.B.F.,

Table 1. FISH Analysis of the Colonic Microbiota at Different Stages of *C. rodentium* and *C. jejuni* Infection

Infection	EUB338 (All Eubacteria)		CFB286 (Bacteroides Class)		GAM42α	(γ-Proteobacteria class)	Calculated	(non-CFB286/ GAM42α)
C. rodentium	C	I	C	I	C	I	C	I
7 days	2.63 (0.17)	0.75 (0.22)***	1.81 (0.23)	0.21 (0.16)***	<0.02	0.10 (0.05)*	0.82 (0.28)	0.54 (0.16)
14 days	2.60 (0.31)	0.99 (0.43)*	1.94 (0.28)	0.52 (0.36)*	<0.02	<0.02	0.66 (0.22)	0.47 (0.23)
28 days	2.50 (0.48)	2.22 (0.42)	1.83 (0.34)	1.76 (0.33)	<0.02	<0.02	0.66 (0.19)	0.46 (0.21)
C. jejuni	C	I	C	I	C	I	C	I
3 days	3.4 (1.2)	3.7 (6.3)	0.5 (0.08)	0.7 (0.3)	0.02 (0.02)	0.2 (0.3)	2.8 (0.09)	2.8 (0.6)
7 days	3.4 (1.2)	3.4 (2.3)	0.5 (0.08)	0.7 (0.3)	0.02 (0.02)	0.03 (0.04)	2.8 (0.09)	2.7 (0.3)
14 days	3.4 (1.2)	2.3 (1.5)	0.5 (0.08)	0.6 (0.3)	0.02 (0.02)	0.04 (0.09)	2.8 (0.09)	1.7 (0.06)
C. rodentium Shifted Baseline Microbiota	C	I	C	I	C	I	C	I
10 days	1.37 (0.38)	0.33 (0.21) *	0.07 (0.04)	0.02 (0.02)	<0.02	0.10 (0.04) *	1.29 (0.04)	0.20 (0.04) *

Numbers represent the mean number of bacterial cells per gram colon × 10^{10} (SD). C = control groups, I = infected groups. *C. rodentium* infection: n = 6 mice in all groups; *p < 10^{-3} two-tailed t test, **p < 10^{-4} two-tailed t test, ***p < 10^{-7} two-tailed t test; each time point was assessed in 3 different experiments with the same outcome. *C. jejuni* infection: n = 4 mice in 3 days infected group, n = 5 mice for other groups; no significant differences were observed between the different groups; 3 days time point was assessed once, 7 days time point was assessed in three different experiments, and 14 days time point was assessed in two different experiments with the same outcome. *C. rodentium* shifted baseline microbiota infection: n = 5 mice for control group, n = 6 mice for infected group; *p < 10^{-3} two tailed t test; time-point was assessed in two different experiments with the same outcome.

unpublished data). *C. jejuni* belongs to the family Campylobacteraceae of the ε-Proteobacteria class in the phylum Proteobacteria.

Examination of the numbers of microbial cells in mouse colons at different stages of *C. jejuni* infection established that while there was a nonsignificant reduction in the total number of colonic bacteria at 7 and 14 dpi, no correlation with *C. jejuni* colonization was observed (Figure 1B). Interestingly, unlike infection with *C. rodentium*, colonization of mice by *C. jejuni* did not result in significant changes in the total number of colonic bacteria despite colonizing to levels comparable to *C. rodentium* (reaching up to 10^8 cfu/g colon). The extent of colonization by *C. jejuni* did not change significantly over the course of the infection, although some variation in numbers was observed.

These results suggested an association between pathogen-induced intestinal inflammation and extensive changes in levels of colonic bacteria. A clear and significant reduction in the total numbers of bacteria was observed over the course of *C. rodentium* colonization and resultant intestinal pathology, while colonization by a foreign enteric pathogen that does not induce inflammation (i.e., *C. jejuni*) alone failed to produce similar changes.

Microbiota Composition Changes during Pathogen-Induced Inflammation, with a Preferential Elimination of a Subset of Microbiota

To examine the possibility of changes in microbiota composition during various stages of infection, we used fluorescence in situ hybridization (FISH) using either a probe specific to bacteria of the Cytophaga-Flavobacter-Bacteroides (CFB) class or a probe targeting γ-Proteobacteria in combination with a general eubacterial probe (Table 1).

Consistent with the above results, FISH analysis indicated that, during *C. rodentium* infection, the total number of bacterial cells was reduced 3-fold relative to uninfected mice at 7 and 14 dpi (Table 1). The majority of rod-shaped bacteria in the colon could be assigned to the CFBs, composing on average 71% of all bacteria in healthy animals but only 28% and 52% of bacteria in samples taken at 7 and 14 dpi, respectively (Table 1). Consequently, bacteria belonging to the CFBs appear to be preferentially eliminated during infection, with an up to 8.5-fold reduction in numbers of CFBs in animals infected for 7 days, compared to CFB numbers in control animals and a 3.5-fold reduction in numbers in animals infected for 14 days (Table 1). In contrast, bacteria that did not hybridize with either CFB or γ-Proteobacteria probes were reduced less than 2-fold (Table 1). At 7 dpi, 14% of bacteria in the mouse colon belonged to the γ-Proteobacteria (Table 1). These numbers closely correlated with the number of *C. rodentium* cells recovered by selective plating on MacConkey agar plates (Figure 1A), and presumably the vast majority of the detected γ-Proteobacteria represented this pathogen. All changes were transient; following resolution of infection, the colonic microbiota composition of infected and healthy mice was indistinguishable (Table 1).

FISH analysis of *C. jejuni*-infected samples demonstrated no significant change in the total number of bacteria during *C. jejuni* colonization (Table 1), which was consistent with the results obtained by SYBR green staining (Figure 1B). Similarly, there were no significant differences

in the proportions of either the Bacteroides (CFB) or the γ-Proteobacteria classes making up the colonic microbiota. There was no significant variation in the proportion of non-CFB/non-γ-Proteobacteria classes making up the microbiota, although clearly in the infected, but not in control samples, *C. jejuni* would account for ~1% of microbiota not detected by FISH; however, an ε-Proteobacteria specific probe was not available. Thus, in the absence of pathogen-induced inflammatory response and during colonization by the non-γ-Proteobacteria bacterium *C. jejuni*, there were no gross alterations in either the total numbers of mouse colonic microbiota or the representation of the CFB or γ-Proteobacteria classes. Since no significant changes were observed, samples from mice infected with *C. jejuni* were not subjected to sequencing analysis.

FISH analysis indicated that over the course of the study, the baseline composition of colonic microbiota of mice has changed (Table 1). While in the initial experiments the CFB bacteria made up the largest proportion of the microbiota (*C. rodentium* infection), in later experiments the balance shifted toward the non-CFB/non- γ-Proteobacteria classes (*C. jejuni* infection). The change occurred in the absence of any changes to mouse housing conditions in our animal facility or in the supplier's rearing and housing practices. We therefore wanted to confirm that the mice with the shifted baseline microbiota composition exhibit the same alterations in the microbiota in response to *C. rodentium* infection as the original mice did. We sampled the microbiota of the shifted mice infected with *C. rodentium* for 10 days (allowing the inflammation to be sufficiently manifested, but prior to initiation of *C. rodentium* clearance). Notably, changes in the microbiota of infected shifted mice were similar to those of the original mice: we observed a significant increase in the absolute numbers and proportion of γ-Proteobacteria, as well as a significant reduction in the total intestinal bacteria as a result of *C. rodentium* infection. Unlike the original mice, however, the shifted mice had a significant decrease in the non-CFB/non-γ-Proteobacteria bacteria (~6.5 fold reduction), but not in the CFB bacteria (~3.5 fold reduction). Both types of mice, however, suffered a significant reduction in the dominant component of the microbiota as a result of *C. rodentium* infection. Overall, the changes of most interest to us—the reduction in the total number of bacteria and the increase in the γ-Proteobacteria—occurred in both sets of mice, demonstrating that pathogen infection effects certain predictable changes in the microbiota, regardless of natural fluctuations in its baseline composition.

To investigate the *C. rodentium*-induced changes observed in the colonic microbiota composition during infection in more detail and to confirm the results described above by an independent method, 16S rRNA sequence analysis was applied. As suggested by previous work (Eckburg et al., 2005; Ley et al., 2005), we found a remarkable diversity of the colonic microbiota at the genus level (Table 2), but could assign all sequences to only three phyla: the Bacteroidetes, the Firmicutes, and the Proteobacteria. Within these phyla, essentially all sequences matched most closely to the orders Bacteroidales, Clostridiales, and Enterobacteriales, respectively. Due to the number of sequences obtained for each sample and the variation between samples, a meaningful examination of the differences between samples on a lower phylogenetic level was not feasible; however, the changes observed were significant at the phylum level.

Consistent with FISH analysis, on average 89% of 16S rRNA sequences obtained from colons of uninfected control animals matched to members of the order *Bacteroidales*, while the majority of the remaining sequences could be assigned to the order *Clostridiales* (Figure 2, Table 2). At 7 dpi, the proportion of 16S rRNA sequences matching the order *Bacteroidales* was significantly decreased to 27%, while the proportion of *Clostridiales* stayed approximately the same with 28%, and 41% of the obtained sequences assigned to *C. rodentium* (Figure 2). At dpi consistent with the initiation of clearance of infection, none of the sequences could be assigned to *C. rodentium*. Although the total number of bacterial cells was still significantly reduced (Figure 1A, Table 1), the relative proportions of bacteria were not significantly different from uninfected control animals (Figure 2; Table 2). At 28 dpi, both the relative number of bacteria and the microbiota composition were indistinguishable from uninfected animals (Figure 2, Table 2).

Although evaluation by SYBR green staining, FISH, and 16S rRNA sequences exhibited variance in the exact values of reduction in total colonic microbes and an increase in the γ-Proteobacteria as a result of *C. rodentium* infection, all methods demonstrated statistically significant changes in the same direction. Similar changes were not observed during infection with *C. jejuni*, which although able to colonize the mouse colon, does not induce inflammation. Thus, it was apparent that colonization by a foreign bacterium alone was not sufficient to perturb the host microbiota, and presumably additional factors, such as host inflammatory response, were required. To further investigate this hypothesis, two models of noninfectious intestinal inflammation were used.

Chemically Induced Intestinal Inflammation Causes Microbiota Changes Resembling Enteropathogenic Infection

The first model used to determine the contribution of intestinal inflammation to the changes observed in colonic microbiota during infection was an intestinal inflammatory model based on oral administration of dextran sodium sulfate (DSS) to C57BL/6 mice. The cytotoxic effects of DSS have been demonstrated to increase intestinal permeability, and the resulting loss in barrier function is believed to allow the translocation of microbial products across the intestinal epithelium where they induce a robust inflammatory response (Kitajima et al., 1999; Mueller and Macpherson, 2006).

When examined by culturing techniques, a major difference between control animals and animals treated with DSS for 7 days was an increase in aerobically culturable bacteria on LB medium, from $3.0 \pm 5.3 \times 10^6$ cfu/g colon in control animals to $4.2 \pm 6.6 \times 10^8$ cfu/g colon in DSS treated animals (mean of 4 experiments with n = 3–6

Table 2. Details of 16S rRNA Sequencing Assignment for the Colonic Microbiota of C57BL/6 Mice at Different Stages of *C. rodentium* Infection

						Group							
						Control				Infected			
Phylum	Class	Order	Family	Genus		Day				Day			
						3	7	14	28	3	7	14	28
Bacteroidetes	Bacteroides (CFB)	Bacteroidales	Porphyromonadaceae	*Tannerella*		26	74	70	63	51	24	53	73
				Unclassified		10	4	1	5	2		4	1
			Unclassified			9	3	2	4	3		8	2
					Subtotal	45	81	73	72	56	24	65	76
Firmicutes	Bacilli	Bacillales	Turicibacteraceae	*Turicibacter*		1	1	1			3		
		Lacto-bacillales	Lacto-bacillaceae	*Lacto-bacillus*									1
			Unclassified					1					
	Clostridia	Clostridiales	Acidamino-coccaceae	*Papillibacter*		2			2	2	3	1	1
			Clostridiaceae	*Anaerotruncus*		2	1			3			
				Bryantella						1	1	1	
				Acetivibrio						1			
				Coprobacillus							4		
				Unclassified		1		3	1	4	3	1	2
			Lachno-spiraceae	*Roseburia*							1		
				Unclassified									1
			Unclassified				2	8					7
		Unclassified				23			10	19	12	8	
	Mollicutes	Anaero-plasmatales	Anaero-plasmataceae	*Anaeroplasma*					1				
	Unclassified					6		2	1	1	1		
					Subtotal	35	4	15	15	31	28	11	12
Proteobacteria	γ-Proteo-bacteria	Entero-bacteriales	Entero-bacteriaceae	*Citrobacter*						1	34		
				Salmonella							1		
				Unclassified							1		
					Subtotal	0	0	0	0	1	36	0	0
Unclassified						3		1		1	2		1
Total						83	85	89	87	89	90	76	89

Naive Bayesian rRNA Classifier Version 1.0, November 2003 and the nomenclatural taxonomy of Garrity and Lilburn, release 6.0 were used for analysis; the confidence threshold is 80%; groups were comprised of 6 mice each; colonic samples from all mice in each group were pooled for genomic DNA extraction and a total of 96 clones per pooled sample were sequenced; C = control group, I = infected group; numbers represent the number of sequences that clustered with each group of bacteria; total is the number of total sequences that could be assigned.

mice per treatment group ± standard deviation, $p = 0.30$ by two-tailed paired t test). Using selective plating on Kanamycin Esculin Azide agar and 16S rRNA sequencing of isolated colonies, it was shown that the vast majority of the aerobic bacteria in DSS-treated animals belong to the *Enterococcus faecalis* species (order Lactobacillales, class Bacilli, phylum Firmicutes). However, they still comprised only a small proportion of the colonic microbiota, as Lactobacillales were not detected by 16S rRNA sequencing of the intestinal microbiota community (Table 3). Other changes in the colonic microbiota were similar to those seen in *C. rodentium*-infected mice (Figures 2 and 3A;

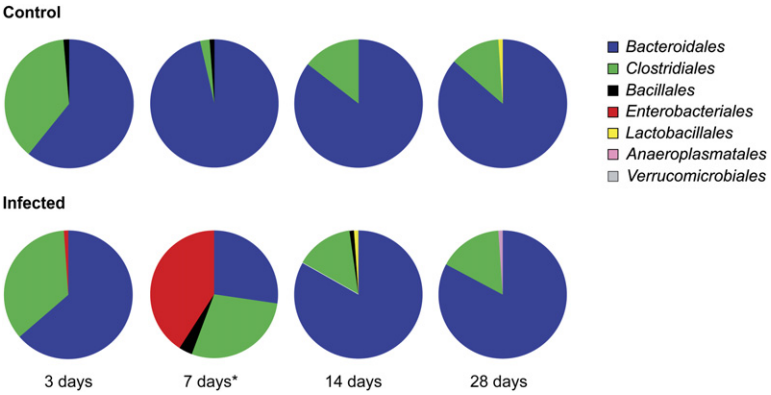

Figure 2. *C. rodentium* Infection Significantly Alters the Intestinal Microbiota Composition, as Assessed by 16S rRNA Analysis

Pie charts represent the relative abundance of the main microbial phyla in the colonic microbiota of mice at different stages of *C. rodentium* infection, as compared to control samples. Phylogenetic orders are represented by different colors. Each group was comprised of 6 mice; colonic samples from all mice in each group were pooled for genomic DNA extraction and a total of 96 clones per pooled sample were sequenced. *$p < 10^{-5}$ for Bacteroidales, Clostridiales, and Enterobacteriales (Audic and Claverie, 1997). Each time point was assessed in three different experiments with the same outcome.

Tables 2 and 3). Using 16S rRNA analysis, the phylum *Bacteroidetes* was found to be selectively eliminated during DSS treatment (Figure 3A; Table 3) and the total number of bacteria decreased, although not as dramatically as during *C. rodentium* infection, to 70% ± 20% that of the control (mean of 4 experiments ± standard deviation, n = 3–6 mice per treatment group, p = 0.08 by two-tailed paired t test).

Chemically Induced Intestinal Inflammation and Host IL-10 Deficiency Promotes Growth of Nonpathogenic *E. coli*

During the course of conducting experiments examining the effect of DSS treatment on mouse colonic microbiota, it was noted that one batch of mice was colonized with detectable low levels of nonpathogenic *E. coli* (identified by selective plating on MacConkey agar plates and 16S rRNA sequencing). Interestingly, when treated with DSS, the numbers of nonpathogenic *E. coli* in these mice increased from $4.7 \pm 3.4 \times 10^4$ per g colon in control animals to $4.7 \pm 6.8 \times 10^8$ per g colon in DSS-treated animals (n = 2–6 mice, p = 0.15 by two-tailed t test), levels that are comparable to those observed in pathogenic *C. rodentium* infection (Figure 1A). This observation prompted us to hypothesize that intestinal inflammation is conducive to overgrowth of Enterobacteriaceae.

To test this hypothesis, the nonpathogenic intestinal *E. coli* isolate described above was introduced into C57BL/6 mice that did not have detectable levels of the nonpathogenic *E. coli* strain, and the microbiota composition of these mice was examined with and without DSS treatment. Consistent with previous results, nonpathogenic *E. coli* levels in DSS-treated animals were comparable to *C. rodentium* levels during infection, while *E. coli* levels in untreated mice were relatively low (Figure 3B). Investigation of the microbiota composition using 16S rRNA analysis showed changes similar to those observed previously; i.e., DSS treatment induced a reduction in the proportion of Bacteroidales from 74% to 60% of total microbiota (Figure 3D; Table 3). However, these microbiota changes were less pronounced than in animals without nonpathogenic *E. coli* (Figure 3A versus 3D, Table 3).

To further test the hypothesis that the changes observed in the microbiota during infection and the overgrowth of Enterobacteriacea are the consequence of the inflammatory host response, we used the IL-10 KO mice that are genetically predisposed to inflammation (Shull et al., 1992; Kuhn et al., 1993). In addition to chemically induced models of mucosal inflammation, genetic models of intestinal inflammation, such as mice deficient in the regulatory cytokines IL-2 (Sadlack et al., 1993), TGF-β (Shull et al., 1992), or IL-10 (Shull et al., 1992; Kuhn et al., 1993), are commonly used to examine the role of inflammation in disease. The absence of the anti-inflammatory cytokine IL-10 results in the development of spontaneous colitis, which is believed to be caused by an aberrant immune reaction to the intestinal microbiota (Shull et al., 1992; Kuhn et al., 1993).

When the nonpathogenic intestinal *E. coli* isolate described above was introduced into the intestine of the IL-10 KO mice, it was able to colonize to very high numbers without the requirement of DSS treatment (Figure 3C). Furthermore, the microbiota composition of uninfected IL-10 KO mice appeared to contain a smaller proportion of Bacteroidales relative to wild-type mice (compare Figure 3E to wild-type controls in Figures 2 and 3A). Proliferation of the non-pathogenic *E. coli* in these mice appeared to displace the bacteria from the Firmicutes phylum, rather than the Bacteroidetes (Figure 3E; Table 3); however, an increase in the proportion of γ-Proteobacteria that was observed was comparable to that in the DSS-treated C57BL/6 mice associated with the nonpathogenic *E. coli* isolate (Figure 3D, Table 3). This further supports the proposition stated above that, regardless of the variations in the baseline composition of the intestinal microbiota, colitis modifies it in such a way as to support colonization by Enterobacteriaceae.

DISCUSSION

Our work tested the hypothesis that enteropathogenic infection and associated intestinal inflammation disrupt the intestinal microbial community, and that the disruption can be attributed to either the colonization by the enteric

Table 3. Details of 16S rRNA Sequencing Assignment for the Colonic Microbiota of C57BL/6 Mice with Chemically Induced Inflammation and in Genetically Predisposed to Inflammation IL-10 KO Mice with and without Nonpathogenic *E. coli* Infection

						C57BL/6 Mice				IL-10 KO mice	
Phylum	Class	Order	Family	Genus		C	DSS	Ec	Ec DSS	C	Ec
Bacteroidetes	Bacteroides (CFB)	Bacteroidales	Porphyro-monadaceae	*Tannerella*		33	23	49			
				Unclassified		13	5	9	52	21	9
			Unclassified			17	8	6	5	20	46
					Subtotal	63	36	64	57	41	55
Firmicutes	Bacilli	Bacillales	Turicibacteraceae	*Turicibacter*		1			1		
	Clostridia	Clostridiales	Acidamino-coccaceae	*Papillibacter*			1	2		1	
				Unclassified					2		
			Clostridiaceae	*Bryantella*							2
				Acetivibrio			1	1			
				Unclassified		3	3	1	7	2	
			Lachnospiraceae	*Butyrivibrio*						1	
				Unclassified		2	4		1	6	6
			Unclassified				21	12	18	34	24
		Unclassified				12				2	2
	Mollicutes	Anaero-plasmatales	Anaero-plasmataceae	*Anaeroplasma*			2				
				Unclassified					1		
	Unclassified					2	4	5	2		
					Subtotal	20	36	21	32	46	34
Proteobacteria	γ-Proteo-bacteria	Entero-bacteriales	Entero-bacteriacea	*Escherichia*							2
				Unclassified					3		
					Subtotal	0	0	0	3	0	2
Verrucomicrobia	Verrucomi-crobiae	Verrucomi-crobiales	Verrucomi-crobiaceae	*Verrucomi-crobium*		1	5				
					Subtotal	1	5	0	0	0	0
Unclassified						1	1	1	1		
Total						85	78	86	93	87	91

Naive Bayesian rRNA Classifier Version 1.0, November 2003 and the nomenclatural taxonomy of Garrity and Lilburn, release 6.0 were used for analysis; the confidence threshold is 80%; C57BL/6 C and DSS groups were comprised of 6 mice each, the rest of the groups were comprised of 3 mice each; colonic samples from all mice in each group were pooled for genomic DNA extraction and a total of 96 clones per pooled sample were sequenced; C = control group, DSS = DSS treatment for 7 days, Ec = *E. coli* infection, Ec DSS = *E. coli* infection with DSS treatment; numbers represent the number of sequences that clustered with each group of bacteria; total is the number of total sequences that could be assigned.

pathogen, the host-mediated inflammatory response, or a combination of both factors.

A major hallmark of diarrheal disease caused by the mouse enteric pathogen *C. rodentium* is a dramatic inflammatory response resulting in colonic mucosal hyperplasia and a local Th1 inflammatory response (Higgins et al., 1999). During the initial phase of induction of host inflammatory response to *C. rodentium*, we saw a significant decrease in the total numbers of colonic bacteria, while *C. rodentium* levels were increasing. The decrease in the total number of colonic bacteria was accompanied by significant alterations in the composition of the intestinal microbiota. Following elimination of *C. rodentium* by the adaptive immune response and subsequent resolution of intestinal inflammation, first the composition of the intestinal microbiota and then the total numbers of bacteria returned to the preinfection state, indicating that the intestinal ecosystem is remarkably robust and can recover

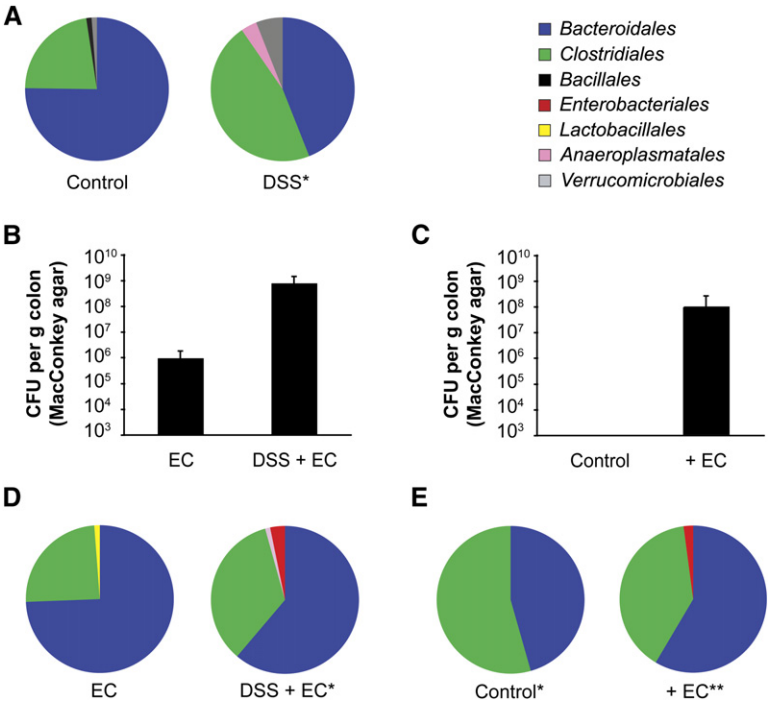

Figure 3. Chemically Induced Inflammation and Genetic Predisposition to Inflammation in Mice Promotes Colonization by Nonpathogenic *E. coli* and Alters the Intestinal Microbiota Composition

(A) Pie charts represent the relative abundance of the main microbial phyla in the colonic microbiota of mice after 7 days of 3% dextran sodium sulfate (DSS) treatment as compared to untreated controls. Each group was comprised of 6 mice; colonic samples from all mice in each group were pooled for genomic DNA extraction and a total of 96 clones per pooled sample were sequenced. *$p < 0.01$ for Bacteroidales and Clostridiales (Audic and Claverie, 1997). Experiment was repeated four times with the same outcome.

(B) Levels of artificially introduced nonpathogenic *E. coli* (cfu enumerated on Mac Conkey plates) with (DSS + EC) and without (EC) DSS treatment for 7 days (n = 3 animals per group with the indicated SD, experiment was repeated three times with the same outcome).

(C) Levels of artificially introduced nonpathogenic *E. Coli* in genetically predisposed to inflammation IL-10 KO mice (control = uninfected IL-10 KO mice; +EC = IL-10 KO mice with artificially introduced nonpathogenic *E. coli*) (n = 3 animals per group with the indicated SD, experiment was repeated twice with the same outcome).

(D) 16S rRNA analysis of the colonic microbiota of animals with artificially introduced nonpathogenic *E. coli* with and without DSS treatment for 7 days. Phylogenetic orders are represented by different colors. Each group was comprised of three mice; colonic samples from all mice in each group were pooled for genomic DNA extraction and a total of 96 clones per pooled sample were sequenced.

(E) 16S rRNA analysis of the colonic microbiota of genetically predisposed to inflammation IL-10 KO mice. Phylogenetic orders are represented by different colors. Each group was comprised of three mice; colonic samples from all mice in each group were pooled for genomic DNA extraction and a total of 96 clones per pooled sample were sequenced. *$p < 0.05$ for Bacteroidales and Clostridiales (Audic and Claverie, 1997) for EC versus DSS + EC groups; **$p < 10^{-7}$ for Bacteroidales and Clostridiales for Control versus +EC groups, ***$p < 10^{-3}$ for Bacteroidales and Clostridiales for +EC versus control wild-type mice (Figure 2, 28 day control) (Audic and Claverie, 1997); DSS versus DSS + EC experiment was conducted three times with the same outcome; Control versus +EC experiment was conducted twice with the same outcome.

quickly even after major disturbances. The relative ability of *C. rodentium* to colonize the intestine during the host inflammatory response may not only be due to the pathogen's ability to intimately attach to the host epithelium (Luperchio and Schauer, 2001), but also to a relatively low sensitivity to host-secreted antimicrobials (Gobert et al., 2004; Vallance et al., 2002), a competitive growth advantage in the altered microenvironment due to the occurrence of watery diarrhea resulting in an increase in water content and potentially oxygen availability (Luperchio and Schauer, 2001), and/or a differential sensitivity to metabolic products secreted by the altered microbial community. Considering that the intestine is a complex ecosystem harboring a very diverse microbial community that is metabolically dependent on the animal host, it is likely that each of these effects will have a major impact on its composition and performance. Thus the underlying mechanism of the observed microbiota changes presented here is likely the result of a multitude of factors, both host and microbial community derived.

Conversely, none of the above described changes were observed when mice were infected with *C. jejuni*, a human diarrheal pathogen that, despite being able to successfully colonize, fails to induce inflammatory response in an immunocompetent murine host (Rinella et al., 2006; Mansfield et al., 2007). Thus, it appeared that colonization of the intestinal tract by a foreign bacterium alone was not sufficient to decrease the total levels of the intestinal microbiota or to significantly alter the proportion of resident bacteria belonging to either the CFB or the γ-Proteobacteria classes. These changes appeared to be attributable to the host-mediated intestinal inflammation.

To further test this hypothesis, we utilized two pathogen-independent models of intestinal inflammation: DSS-induced colitis in C57BL/6 mice (Wirtz et al., 2007) and IL-10 KO mice that are genetically predisposed to inflammation (Shull et al., 1992; Kuhn et al., 1993).

Both IL-10 KO mice and C57BL/6 mice with DSS-induced colitis could be easily colonized with nonpathogenic intestinal *E. coli* isolate when it was introduced into their gastrointestinal tract. The nonpathogenic *E. coli* isolate was able to colonize to levels comparable to those of *C. rodentium* during *C. rodentium* infection of C57BL/6 mice. In mice with DSS-induced colitis, an

increase in the colonization by *Enterococcus faecalis* was also observed. *Enterococcus faecalis* are facultative anaerobic bacteria that may be benefiting from the potentially increased oxygen availability in the inflamed intestine, in the manner similar to *C. rodentium* and nonpathogenic *E. coli*. Further, chemically induced inflammation caused changes in the intestinal microbiota composition similar to those observed in response to *C. rodentium* infection.

The total levels of intestinal bacteria in mice with DSS-induced colitis were reduced, although not as dramatically as during *C. rodentium* infection. Similarly, a slight reduction in the total numbers of colonic bacteria was observed during noninflammatory infection with *C. jejuni*. It thus appears that, although inflammation alone is sufficient to promote the overgrowth of Enterobacteriaceae, both inflammation and the presence of an invading pathogen are necessary for the decrease in the total numbers of intestinal bacteria to reach its maximal magnitude.

In all of the inflammation models tested, infection, chemical assault, and genetic predisposition, an overgrowth of Enterobacteriaceae (and of *Enterococcus faecalis* in the DSS-induced colitis model) was observed, indicating that inflammation-induced changes in the intestinal microbiota support colonization by aerotolerant bacteria. Thus, the response of the intestinal microbiota to all inflammation models appeared similar, implying that the underlying mechanisms may be nonspecific. It also appears that the inflammatory response triggered by the invading pathogen functions to enhance its colonization, thereby further facilitating its virulence.

The results of this study are not only interesting in the field of infectious diseases, shedding light on colonization practices of enteric pathogens and also looking at the relative advantage of host response to the host and the pathogen; they are also of interest in the context of inflammatory bowel disease (IBD). Crohn's disease and ulcerative colitis are two intestinal inflammatory conditions referred to by the umbrella term IBD. IBD is a disease of elusive etiology, and although many potential triggers have been identified, no unequivocal mechanism of pathogenesis can be proposed. Both genetic and environmental factors have been implicated in the pathogenesis of IBD, and one attractive hypothesis is that the condition develops as a result of an overly aggressive T-cell-mediated immune response to certain commensal bacteria (Sartor, 2006, 2007). The development of the overly aggressive immune response to the intestinal microbiota may be a result of genetic hyperimmunoreactivity, of a dysbiosis in the intestinal microbial community that promotes overgrowth of bacteria that are more stimulatory to the intestinal immune system, or a combination of both of these factors (Strober et al., 2007). Many researchers have reported that the microbial populations in the intestinal tract of IBD patients are different from those of healthy individuals (Gophna et al., 2006; Martinez-Medina et al., 2006; Wang et al., 2007; Andoh et al., 2007); however, the question remains as to whether the differences arise prior to the initiation of the disease process or are the result of hyperactive immune response to the previously balanced microbiota. In all of these studies, an increase in the proportion of nonpathogenic γ-Proteobacteria (Enterobacteriaceae family) and/or presence of pathogenic γ-Proteobacteria was observed in IBD patients relative to healthy controls. These findings are highly reminiscent of the changes in the colonic microbiota that were observed in the present study. Therefore, it would appear that the launch of an inflammatory response by the host could be the initiating factor in the dysregulation of the intestinal microbiota balance and instigation of the persistent inflammatory state of IBD. This is further supported by recent findings that a deficiency of NEMO (a negative regulator of proinflammatory transcription factor NF-κB) is sufficient to initiate a state of chronic inflammation in the murine intestinal tract, although TLR activation by the intestinal microbiota was required for the initiation of proinflammatory response (Nenci et al., 2007).

We have demonstrated that the intestinal microbial community changes in predictable ways in response to host-mediated intestinal inflammation. The observed changes are drastic and significant at the phylum level. Although at the depth of sequencing that we have undertaken the changes observed at the genus and species levels were not statistically significant, these data will prove a stimulus to more detailed work examining subtle changes in composition. Further studies should focus on deciphering the changes that occur at genus and species level in order to illuminate the details of the mechanisms behind the changes described herein.

EXPERIMENTAL PROCEDURES

Mouse Experiments

Wild-type C57BL/6 mice and IL-10 KO mice Bl6.129P2-IL10tm1Cgn/J (Jackson Laboratory, Bar Harbor, Maine) were housed in the animal facility at the University of British Columbia in direct accordance with guidelines drafted by the University of British Columbia's Animal Care Committee and the Canadian Council on the Use of Laboratory Animals. Mice were fed a standard sterile chow diet (Laboratory Rodent Diet 5001, Purina Mills, St. Louis, Missouri) ad libidum throughout the experiments. For infection studies, mice were orally infected with either wild-type *Citrobacter rodentium* DBS100 (Schauer and Falkow, 1993), a wild-type *Campylobacter jejuni* strain 81–176, originally isolated from a diarrheic patient (Korlath et al., 1985), or a natural nonpathogenic intestinal *E. coli* isolated during the course of these studies. The intestinal *E. coli* isolate was characterized through selective plating on McConkey agar and 16S rRNA sequencing and did not cause diarrheal disease in wild-type or IL-10 KO mice; it was further designated nonpathogenic as mice from Jackson Laboratory are guaranteed to be free of specific pathogens. For chemically induced inflammation studies, mice were given 3% dextran sodium sulfate (M.W. 36,000–50,000, MP Biomedicals) ad libidum for 7 days following regular water for the remainder of the experiments. Mice were sacrificed at the indicated time points and whole colon tissues including feces were weighed and then homogenized in 1 ml PBS. To determine the degree of bacterial colonization, serial dilutions of colon homogenates of 3–6 mice per treatment group (number of mice per group in each experiment is indicated in the Results section or the figure legends) were plated on LB, MacConkey (EMD chemicals), or Kanamycin Esculin Azide (EMD chemicals) agar plates and colony-forming units (cfus) were determined after O/N incubation at 37°C. Statistical analysis was carried out using a two-tailed, two-sample equal variance t test.

Fluorescence Microscopy

A 1:10 dilution of each colon homogenate was stored in 3.7% formalin at 4°C until use. For each treatment, the colonic microbiota of 3–6 mice was analyzed, with the exact number of mice per group in each experiment indicated in the Results section and the figure legends. Statistical analysis was carried out using a two-tailed, two-sample equal variance t test for data describing variation within an experiment and a two-sample paired t test for variation between experiments. All quantifications were carried out blind; i.e., the observer had no knowledge of the source of the specimen analyzed.

SYBR Green DNA Staining

Approximately 10 μl of a 1:100 diluted samples were filtered onto Anodisc 25 filters (Whatman International Ltd) with a pore size of 0.2 μM and 2.5 cm diameter. After complete drying, each sample was stained with 0.25 μl SYBR green (Invitrogen) in 100 μl PBS for 15 min in the dark. Filters were dried and mounted on glass slides with 30 μl antifade solution (50% PBS, 50% glycerol, 0.1% p-phenylenediamine) and viewed with a Zeiss Axioskop 2 microscope. Three to five fields per disc were randomly chosen, the number of cells counted and averaged.

FISH Hybridizations

Approximately 5 μl of the 1:10 diluted sample stored in formalin were diluted in 1 ml PBS and filtered onto a polycarbonate membrane filter (Nucleopore Track-Etch Membrane, Whatman International Ltd). The samples were dehydrated on the filter by soaking for 3 min each in 50%, 80% and 100% EtOH. After air drying, the filter was submersed in 100 μl hybridization solution (0.9 M NaCL, 0.1 M TRIS pH 7.2, 30% Formamide, 0.1% SDS) containing 250 ng each of the general EUB338 probe (5′-GCT GCC TCC CGT AGG AGT-3′) (Amann et al., 1990) fluorescently labeled with Texas red, and 250 ng of either CFB286 probe (5′-TCC TCT CAG AAC CCC TAC-3′) (Weller et al., 2000) or GAM42a probe (5′-GCC TTC CCA CAT CGT TT-3′) (Manz et al., 1992) labeled with fluorescein. The nonsense Texas-red-labeled probe NON338 (5′-ACT CCT ACG GGA GGC AGC-3′) (Wallner et al., 1993) was used as a negative hybridization control. The hybridization was carried out overnight at 37°C in the dark. After incubation, the filters were washed for 15 min at 37°C on a shaker first in 15 ml hybridization solution (0.9 M NaCL, 0.1 M TRIS pH 7.2, 30% Formamide, 0.1% SDS) and then in 15 ml buffer (0.9 M NaCL, 0.1 M TRIS pH 7.2). After complete drying, the filters were mounted on microscope slides with 10 μl antifade solution (50% glycerol, 50% PBS, 0.1% p-phenylenediamine) and viewed and counted as described above. In general, the bacterial concentration in all samples determined by FISH was lower than those determined by SYBR green stain, which is likely due to loss of bacteria during hybridization and washing steps.

Sequence Analysis

Colon homogenates of all animals in each experimental group were pooled and stored at –80°C until use. The exact number of animals used is indicated in the Results section and the figure legends. DNA extractions were carried out using DNAeasy kit according to the manufacturer's protocol with overnight lysis (QIAGEN). Whole 16S rRNA was PCR-amplified using the universal bacterial primers 27f (5′ AGA GTT TGA TCM TGG CTC AG) and 1492r (5′ TAC GGY TAC CTT GTT ACG ACT T 3′) (Lane, 1991) with an annealing temperature of 48°C and cloned using the TOPO TA cloning kit (Invitrogen). Ninety-six individual colonies per sample were picked and each grown in 100 μl LB medium containing 50 μg/ml ampicillin. Sequencing was carried out at LARK Technologies, Inc., Houston, TX and Macrogen, Korea. Sequences were manually checked for accuracy and edited using Sequencher software version 4.5. Clone inserts were either sequenced from both ends, the sequences aligned, and the full 1.5 kb sequence analyzed (Figure 2; Table 2) or sequenced from one end and an approximately 600–900 bp sequence was used for further analysis (Figure 3; Table 3). Ribosomal Database Project (RDP) II Release 9 (http://rdp.cme.msu.edu/) was used for assignment of individual sequences to the taxonimical hierarchy of Bergey's Manual of Systematic Bacteriology (Cole et al., 2005) using a naive Bayesian rRNA classifier. Significance values for differences between sequence libraries were determined using RDP Library Compare (http://rdp.cme.msu.edu/comparison/comp.jsp) which is based on Audic and Claverie (1997) or a standard test of two approximately normally distributed population proportions with the p value estimated from the z critical value.

For species identification, the DNA of isolated colonies was extracted, 16S rRNA PCR amplified and sequenced and analyzed as above.

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REFERENCES

Aebischer, T., Fisher, A., Walduck, A., Schlotelburg, C., Lindig, M., Schreiber, S., Meyer, T., Bereswill, S., and Gobel, U. (2006). Vaccination prevents *Helicobacter pylori*-induced alterations of the gastric flora in mice. FEMS Immunol. Med. Microbiol. *46*, 221–229.

Alakomi, H.L., Skytta, E., Saarela, M., Mattila-Sandholm, T., Latva-Kala, K., and Helander, I.M. (2000). Lactic acid permeabelizes gram negative bacteria by disrupting the outer membrane. Appl Environ. Microbiol. *66*, 2001–2005.

Amann, R.I., Binder, B.J., Olson, R.J., Chisholm, S.W., Devereux, R., and Stahl, D.A. (1990). Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. Appl. Environ. Microbiol. *56*, 1919–1925.

Andoh, A., Sakata, S., Koizumi, Y., Mitsuyama, K., Fujiyama, Y., and Benno, Y. (2007). Terminal restriction fragment length polymorphism analysis of the diversity of fecal microbiota in patients with ulcerative colitis. Inflamm. Bowel Dis. *13*, 955–962.

Audic, S., and Claverie, J.M. (1997). The significance of digital gene expression profiles. Genome Res. *7*, 986–995.

Christensen, H.R., Frokiar, H., and Pestka, J.J. (2002). *Lactobacilli* differentially modulate expression of cytokines and maturation surface markers in murine dendritic cells. J. Immunol. *168*, 171–178.

Coker, A.O., Isokpehi, R.D., Thomas, B.N., Amisu, K.O., and Obi, C.L. (2002). Human campylobacteriosis in developing countries. Emerg. Infect. Dis. *8*, 237–244.

Cole, J.R., Chai, B., Farris, R.J., Wang, Q., Kulam, S.A., McGarrell, D.M., Garrity, G.M., and Tiedje, J.M. (2005). The Ribosomal Database Project (RDP-II): Sequences and tools for high-throughput rRNA analysis. Nucleic Acids Res. *1*, D294–D296.

Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S.R., Nelson, K.E., and Relman, D.A. (2005). Diversity of the human intestinal microbial flora. Science *308*, 1635–1638.

Gibson, J.R., and Roberfroid, M.B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr. *125*, 1401–1412.

Gobert, A.P., Cheng, Y., Akhtar, M., Mersey, B.D., Blumberg, D.R., Cross, R.K., Chaturvedi, R., Drachenberg, C.B., Boucher, J.L., Hacker, A., et al. (2004). Protective role of arginase in a mouse model of colitis. J. Immunol. *173*, 2109–2117.

Gomes, D.A., Souza, A.M., Lopes, R.V., Nunes, A.C., and Nicoli, J.R. (2006). Comparison of antagonistic ability against enteropathogens by G+ and G- anaerobic dominant components of human fecal microbiota. Folia Microbiol. (Praha) *51*, 141–145.

Gophna, U., Sommerfeld, K., Gophna, S., Doolittle, W.F., and Veldhuyzen van Zanten, S.J. (2006). Differences between tissue-associated intestinal microfloras of patients with Crohn's disease and ulcerative colitis. J. Clin. Microbiol. *44*, 4136–4141.

Higgins, L.M., Frankel, G., Douce, G., Dougan, G., and MacDonald, T.T. (1999). *Citrobacter rodentium* infection in mice elicits a mucosal Th1 cytokine response and lesions similar to those in murine inflammatory bowel disease. Infect. Immun. *67*, 3031–3039.

Hooper, L.V., Midtvedt, T., and Gordon, J.I. (2002). How host-microbial interactions shape the nutrient environment of the mammalian intestine. Annu. Rev. Nutr. *22*, 283–307.

Imaoka, A., Matsumoto, S., Setoyama, H., Okada, Y., and Umesaki, Y. (1996). Proliferative recruitment of intestinal intraepithelial lymphocytes after microbial colonization of germ-free mice. Eur. J. Immunol. *26*, 945–948.

Kitajima, S., Takuma, S., and Morimoto, M. (1999). Changes in colonic mucosal permeability in mouse colitis induced with dextran sulfate sodium. Exp. Anim. *48*, 137–143.

Korlath, J.A., Osterholm, M.T., Judy, L.A., Forfang, J.C., and Robinson, R.A. (1985). A point-source outbreak of campylobacteriosis associated with consumption of raw milk. J. Infect. Dis. *152*, 592–596.

Kuehl, C.J., Wood, H.D., Marsh, T.L., Schmidt, T.M., and Young, V.B. (2005). Colonization of the cecal mucosa by *Helicobacter hepaticus* impacts the diversity of the indigenous microbiota. Infect. Immun. *73*, 6952–6961.

Kuhn, R., Lohler, J., Rennick, D., Rajewsky, K., and Muller, W. (1993). Interleukin-10-deficient mice develop chronic enterocolitis. Cell *75*, 263–274.

Lane, D.J. (1991). 16S/23S rRNA sequencing. In Nucleic Acid Techniques in Bacterial Systematics, E. Stackebrandt and M. Goodfellowеds, eds. (Chichester: John Wiley &Sons), pp. 115–175.

Ley, R.E., Backhed, F., Turnbaugh, P., Lozupone, C.A., Knight, R.D., and Gordon, J.I. (2005). Obesity alters gut microbial ecology. Proc. Natl. Acad. Sci. USA *102*, 11070–11075.

Luperchio, S.A., and Schauer, D.B. (2001). Molecular pathogenesis of *Citrobacter rodentium* and transmissible murine colonic hyperplasia. Microbes Infect. *3*, 333–340.

Mansfield, L.S., Bell, J.A., Wilson, D.L., Murphy, A.J., Elsheikha, H.M., Rathinam, V.A., Fierro, B.R., Linz, J.E., and Young, V.B. (2007). C57BL/6 and congenic interleukin-10-deficient mice can serve as models of Campylobacter jejuni colonization and enteritis. Infect. Immun. *75*, 1099–1115.

Manz, W., Amann, R., Ludwig, W., Wagner, M., and Schleifer, K.H. (1992). Phylogenetic oligodeoxynucleotide probes for the major subclasses of Proteobacteria: Problems and solutions. Syst. Appl. Microbiol. *15*, 593–600.

Martinez-Medina, M., Aldeguer, X., Gonzalez-Huix, F., Acero, D., and Garcia-Gil, L.J. (2006). Abnormal microbiota composition in the ileocolonic mucosa of Crohn's disease patients as revealed by polymerase chain reaction-denaturing gradient gel electrophoresis. Inflamm. Bowel Dis. *12*, 1136–1145.

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.

Mueller, C., and Macpherson, A.J. (2006). Layers of mutualism with commensal bacteria protect us from intestinal inflammation. Gut *55*, 276–284.

Nenci, A., Becker, C., Wullaert, A., Gareus, R., van Loo, G., Danese, S., Huth, M., Nikolaev, A., Neufert, C., Madison, B., et al. (2007). Epithelial NEMO links innate immunity to chronic intestinal inflammation. Nature *446*, 557–561.

Reid, G., Howard, J., and Gan, B.S. (2001). Can bacterial interference prevent infection? Trends Microbiol. *9*, 424–428.

Rinella, E.S., Eversley, C.D., Carroll, I.M., Andrus, J.M., Threadgill, D.W., and Threadgill, D.S. (2006). Human epithelial-specific response to pathogenic Campylobacter jejuni. FEMS Microbiol. Lett. *262*, 236–243.

Sadlack, B., Merz, H., Schorle, H., Schimpl, A., Feller, A.C., and Horak, I. (1993). Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene. Cell *75*, 253–261.

Sansonetti, P.J. (2004). War and peace at mucosal surfaces. Nat. Rev. Immunol. *4*, 953–964.

Sartor, R.B. (2006). Mechanisms of Disease: Pathogenesis of Crohn's disease and ulcerative colitis. Nat. Clin. Pract. Gastroenterol. Hepatol. *7*, 390–407.

Sartor, R.B. (2007). Bacteria in Crohn's disease: Mechanisms of inflammation and therapeutic implications. J. Clin. Gastroenterol. *41*, S37–S43.

Schauer, D.B., and Falkow, S. (1993). Attaching and effacing locus of a *Citrobacter freundii* biotype that causes transmissible murine colonic hyperplasia. Infect. Immun. *61*, 2486–2492.

Servin, A.L. (2004). Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. FEMS Microbiol. Rev. *28*, 405–440.

Shi, H.N., and Walker, A. (2004). Bacterial colonization and the development of intestinal defences. Can. J. Gastroenterol. *18*, 493–500.

Shull, M.M., Ormsby, I., Kier, A.B., Pawlowski, S., Diebold, R.J., Yin, M., Allen, R., Sidman, C., Proetzel, G., Calvin, D., et al. (1992). Targeted disruption of the mouse transforming growth factor-beta 1 gene results in multifocal inflammatory disease. Nature *359*, 693–699.

Silva, S.H., Vieira, E.C., Dias, R.S., and Nicoli, J.R. (2001). Antagonism against Vibrio cholerae by diffusible substances produced by bacterial components of the human faecal microbiota. J. Med. Microbiol. *50*, 161–164.

Sprunt, K., and Leidy, G. (1988). The use of bacterial interference to prevent infection. Can. J. Microbiol. *34*, 332–338.

Strober, W., Fuss, I., and Mannon, P. (2007). The fundamental basis of inflammatory bowel disease. J. Clin. Invest. *117*, 514–521.

Vallance, B.A., Deng, W., De Grado, M., Chan, C., Jacobson, K., and Finlay, B.B. (2002). Modulation of inducible nitric oxide synthase expression by the attaching and effacing bacterial pathogen citrobacter rodentium in infected mice. Infect. Immun. *70*, 6424–6435.

Wallner, G., Amann, R., and Beisker, W. (1993). Optimizing fluorescent in situ hybridization with rRNA-targeted oligonucleotide probes for flow cytometric identification of microorganisms. Cytometry *14*, 136–143.

Wang, M., Molin, G., Ahrne, S., Adawi, D., and Jeppsson, B. (2007). High proportions of proinflammatory bacteria on the colonic mucosa in a young patient with ulcerative colitis as revealed by cloning and sequencing of 16S rRNA genes. Dig. Dis. Sci. *52*, 620–627.

Whary, M.T., Danon, S.J., Feng, Y., Ge, Z., Sundina, N., Ng, V., Taylor, N.S., Rogers, A.B., and Fox, J.G. (2006). Rapid onset of ulcerative typhlocolitis in B6.129P2–IL10tm1Cgn (IL-10−/−) mice infected with Helicobacter trogontum is associated with decreased colonization by altered Schaedler's flora. Infect. Immun. *74*, 6615–6623.

Weller, R., Glockner, F.O., and Amann, R. (2000). 16S rRNA-targeted oligonucleotide probes for the in situ detection of members of the phylum Cytophaga-Flavobacterium-Bacteroides. Syst. Appl. Microbiol. *23*, 107–114.

Wirtz, S., Neufert, C., Weigmann, B., and Neurath, M.F. (2007). Chemically induced mouse models of intestinal inflammation. Nat. Protoc. *2*, 541–546.

Xu, J., and Gordon, J.I. (2003). Inaugural Article: Honor thy symbionts. Proc. Natl. Acad. Sci. USA *100*, 10452–10459.