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Correlation of Cecal Microflora of HLA-B27 Transgenic Rats with Inflammatory Bowel Disease

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Transgenic rats with a high level of expression of the human major histocompatibility complex class I molecule HLA-B27 develop chronic inflammatory bowel disease (IBD) and arthritis. Assessment of the cecal microflora showed a rise in numbers of *Escherichia coli* and *Enterococcus* spp., corresponding to the presence and severity of IBD in these rats.

The role of bacteria in Crohn's disease and ulcerative colitis has been of intense interest for four decades, but no single bacterial pathogen has been identified. Current theory suggests that some aspect of the normal microflora may mediate the chronic inflammatory response characteristic of inflammatory bowel disease (IBD). Consistent with this, a number of occurrences of IBD that are seen in rodent models involving conventionally housed animals are prevented by raising the animals in a germfree environment (3). Among the models of IBD dependent upon the presence of intestinal bacteria is the enterocolitis that develops in HLA-B27 transgenic rats (8, 11). Here we report quantitative and qualitative characterization of changes in the bacterial populations of the cecum that correlate with the presence and severity of colitis in this animal model of IBD.

Male and female rats from B27 transgenic disease-prone and disease-resistant lines from separate cages and animal housing rooms and nontransgenic littermates were used (4, 9, 10). Clinical disease was scored as described previously (1). Upon sacrifice, the cecum was excised and snap frozen, and the adjacent proximal colon was processed for histology (4). Previous studies have documented that the freezing process results in a modest decrease in total counts (0.5 $\log_{10}$ CFU) and no qualitative differences in the major groups isolated. Frozen ceca were thawed in an anaerobic chamber. An aliquot was used to determine dry weight. Dilutions of 10^{-2} to 10^{-8} in sterile saline were made from a second aliquot and plated onto both selective and nonselective microbiologic media (6, 7). Plates were incubated under anaerobic or aerobic conditions at 37°C for 48 h. Colonies were counted, and isolates of representative colony types were identified. All counts were reported as $\log_{10}$ CFU per gram (dry weight) for facultative and obligately anaerobic organisms. All microbiologic assessment was conducted without knowledge of clinical or histologic status of the animals. Correlations were sought between the microbiologic findings and the severity of clinical disease (severe, intermediate, and healthy), histologic score (proximal colon score range, 0 to 4 [8]), and quantitative histologic ulcer score (0 to 4).

Representative *Escherichia coli* isolates were probed with pCVD434 for the presence of the toxin-associated *eae* gene and with pJPN16 for the presence of the EAF locus (5). Attachment of *E. coli* was evaluated using HEP-2 cells, and Vero cell cytotoxicity was assayed as described previously (2).

The ceca from 29 rats were evaluated (6 healthy, with a mean age of 287 days; 12 with intermediate disease, with a mean age of 210 days; and 11 with severe disease, with a mean age of 158 days). Quantitative counts are presented in Table 1. Rats with severe disease had somewhat higher numbers of aerobic and facultative organisms (mean $\log_{10}$CFU, 9.51 $\pm$ 0.17) than either healthy rats or rats with intermediate disease. The total counts of anaerobic organisms (anaerobic counts) for healthy animals were approximately 0.5 log unit lower than for animals with either severe or intermediate disease (mean of 9.63 $\pm$ 0.27 versus 10.02 $\pm$ 0.16 and 10.23 $\pm$ 0.23, respectively). By one-way analysis of variance, neither the aerobic nor anaerobic total counts revealed significant differences among the three groups. While there was a significant correlation between age and colon score ($P < 0.001$), no correlation between age and total aerobic or anaerobic counts was observed. A significant correlation was observed between age and *Enterococcus* populations ($P = 0.028$); however, no correlation was observed between age of animals and the *E. coli* population. The total numbers of enterococci revealed a significant difference between rats with severe disease and healthy rats (mean $\log_{10}$ CFU, 9.37 $\pm$ 0.2 versus 8.13 $\pm$ 0.57, respectively [$P < 0.045$]). Those with intermediate disease (mean, 8.67 $\pm$ 0.26) were not significantly different from the other two groups. The most striking difference was seen in the counts for *E. coli*. Healthy animals had relatively modest numbers (mean, 5.89 $\pm$ 0.44; range, 4.6 to 7.14), whereas the rats with severe disease (mean, 8.44 $\pm$ 0.21; range, 7.5 to 9.7) and those with intermediate disease (mean, 7.85 $\pm$ 0.41; range, 5.49 to 10.31) had substantially higher counts ($P < 0.008$ for all groups, $P < 0.0001$ for healthy versus severe disease, and $P < 0.01$ for healthy versus intermediate disease; $r = 0.65$ and $P < 0.05$ for *E. coli* count versus colon score). The increase in the total counts for facultative gram-negative organisms for animals with intermediate or severe disease was almost entirely due to the increase in the numbers of *E. coli* present ($r = 0.94$, $P < 0.001$). Assays of representative *E. coli* isolates from the three groups of rats for the presence of a cytopathic effect on Vero cells, the presence of the enterotoxin-associated *eae* gene and EAF locus, and attachment to the HEP-2 cell line were all negative.

The qualitative microbiologic data revealed certain trends (Table 2). The frequencies of isolation for the various listed genera were similar irrespective of group. Total counts for gram-

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TABLE 1. Quantitative bacterial counts in ceca of HLA-B27 transgenic rats

Measurement	Value(s) for rats with indicated status		
	Healthy	Intermediate disease	Severe disease
No. of rats	6	12	11
Colon histology score	0.67	3.08	3.09
Colon ulceration score	0.00	0.67	3.55
No. of:			
Aerobes	8.69 ± 0.45 (7.52–10.4)[a]	9.12 ± 0.20 (8.23–10.5)	9.51 ± 0.17 (8.62–10.45)
Anaerobes	9.63 ± 0.27 (8.66–10.25)	1.23 ± 0.11 (9.27–10.79)	10.02 ± 0.16 (8.7–10.58)
Enterococcus	8.13 ± 0.57 (6.91–10.4)	8.67 ± 0.26 (6.65–9.98)	9.37 ± 0.20 (8.26–10.45)
E. coli	5.89 ± 0.44 (4.6–7.14)	7.85 ± 0.41 (5.49–10.31)	8.44 ± 0.21 (7.5–9.7)

[a] Mean ± standard error of the mean (range) $\log_{10}$ CFU/gram of cecal contents.

positive facultative organisms, gram-negative facultative organisms, and gram-positive anaerobes were higher for the intermediate and severe disease groups than for the healthy controls. The total counts for facultative gram-negative and gram-positive organisms are explained by the increase in numbers of *E. coli* and *Enterococcus* spp., respectively (see above). The increase in gram-positive anaerobe counts can be explained by increased *Lactobacillus* counts ($r = 0.69$ versus counts for gram-positive anaerobes, $P < 0.05$).

The present study describes initial characterization of the cecal microflora of both healthy animals and HLA-B27 transgenic animals with obvious IBD and documents specific increases in two populations, *E. coli* and *Enterococcus*. It remains to be determined whether these two increases are causally related and their relationship to previous data from reconstitution experiments with germfree rats, which implicated *Bacteroides vulgatus* (8). However, both *E. coli* and *Enterococcus* are pathogens once they gain access to otherwise sterile sites, and both can elaborate products that are toxic to mammalian cells. It has also been shown that human patients with IBD have increased antibody to specific bacterial serotypes, such as *E. coli* O:14, when compared to healthy controls (12). Evaluation of *E. coli* isolates in this study for the presence of traditional enterotoxin activity was negative. However, the possibility that other factors produced by this organism play a role in inducing or sustaining inflammation cannot be ruled out. A possible synergistic relationship between *E. coli* and *Enterococcus* with respect to intestinal inflammation also remains to be considered.

TABLE 2. Frequency of isolation of various groups of bacteria from HLA-B27 rats

Group	Values for rats with indicated status[a]		
	Healthy ($n = 6$)	Intermediate disease ($n = 12$)	Severe disease ($n = 11$)
Gram-negative facultative organisms	6 (6.45)	11 (7.95)	11 (8.47)
Gram-positive facultative organisms	6 (8.42)	12 (8.96)	11 (9.38)
Gram-negative anaerobes	6 (9.03)	12 (9.47)	11 (9.00)
Gram-positive anaerobes	6 (9.08)	12 (9.87)	11 (9.82)
Bacteroides	6 (9.00)	12 (9.33)	11 (8.97)
Prevotella	2 (8.62)	6 (9.61)	5 (9.20)
Lactobacillus	6 (9.00)	12 (9.58)	11 (9.59)
Clostridium	2 (6.81)	1 (9.67)	1 (5.78)
Proteus	2 (7.02)	5 (6.91)	6 (7.44)

[a] Number of rats from which organisms were isolated (mean count, $\log_{10}$ CFU/gram [dry weight]).

Although the observations made during this study involved only a modest number of non-age-matched animals, continued study of the HLA-B27 rats is warranted and should help more clearly define the characteristics of the gut bacterial population responsible for the predictable occurrence of bowel inflammation in this animal model of human IBD (3). Ongoing quantitative microbiologic studies of the HLA-B27 rats using age-matched controls and larger group sizes are currently in progress.

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