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Comparison of *Bacteroides vulgatus* Strains in the Enhancement of Experimental Ulcerative Colitis

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Strains of *Bacteroides vulgatus* from a variety of sources were tested for their abilities to enhance the inflammatory response in an experimental model for ulcerative colitis. Although there were considerable differences noted in inflammatory responses when guinea pigs were immunized with the various strains, there did not appear to be any correlation between the source of the isolates and the severity of the carrageenan-induced lesions. Strains from patients with ulcerative colitis were no more active in the model system than were strains from patients with antibiotic-associated colitis or strains from a healthy human source. The antibody titer to the strain used for immunization did not correlate with the severity of the cecal ulcerations, as determined by histopathologic evaluation.

An experimental guinea pig model simulating many of the histopathologic features of ulcerative colitis was first described in 1969 (9). Cecal and large-bowel ulcerations were induced in this animal species by the administration of solutions of degraded carrageenan for 3 to 4 weeks in the drinking water. Histologic changes noted in experimental animals included crypt abscesses, epithelial thinning, polymorphonuclear cells in the lamina propria, superficial ulcerations in the cecum and large intestine, and loss of crypts (2, 9). The inducing agent, carrageenan, is a sulfated polysaccharide extracted from red seaweeds such as *Eucheuma spinosum* and does not appear to play any role in the development of the human disease (3). Although it is an effective inducer of the experimental disease in guinea pigs, other sulfated polysaccharides have been reported to be effective inducing agents as well (9). Previous studies in this laboratory have used this model to determine whether carrageenan per se provokes the ulcerations in guinea pigs (7). It has been shown that conventional guinea pigs uniformly develop cecal ulcerations when placed on a regimen of 5% degraded carrageenan for 21 days, but germfree guinea pigs lacking any bacterial microflora do not develop cecal ulcerations when maintained on carrageenan for up to 6 months (6). Further experimentation with gnotobiotic guinea pigs identified an organism, *Bacteroides vulgatus*, which appears to provoke cecal ulcerations in gnotobiotic guinea pigs with or without the administration of degraded carrageenan (5). Subsequent studies were directed at defining the role of *B. vulgatus* in the experimental disease process. It has been shown that immunization of guinea pigs with *B. vulgatus* before both the administration of carrageenan and the feeding of viable *B. vulgatus* results in a more rapid development of ulcerations (8). Immunization with a phenotypically similar organism, *Bacteroides fragilis*, does not induce a more pronounced inflammatory response after 21 days of carrageenan administration (8). It was also noted that carrageenan administration to immunized animals without the feeding of viable *B. vulgatus* does not significantly alter the results when compared with carrageenan administration to nonimmune animals (4). An additional study has shown that the immune enhancement of the experimental disease process can be adoptively transferred to naive recipients with spleen cells from actively immunized animals but not with immune serum (8). More important, it was also noted that the immune enhancement noted with *B. vulgatus* appears to have strain specificity, since a strain from a non-inflammatory bowel disease (IBD) source does not provoke the same response as strains from animals with IBD or a patient with IBD (8). The present study summarizes tests in this animal model system of additional strains of *B. vulgatus* from a variety of sources.

Animals were immunized with Formalin-killed whole cells of each test strain by a previously described immunization regimen (4). After immunization, animals were bled for measurement of homologous antibody to the immunization strain. Each group of animals and a nonimmune control group were then placed on a 28-day regimen of 5% degraded carrageenan and daily feedings of 10^9 CFU per guinea pig of viable homologous bacterial cells. At the end of the experiment, all animals were killed, and the ileum, cecum, large intestine, and rectum were removed and fixed in 10% neutral buffered Formalin. Tissues were processed by standard techniques, and slides were encoded for evaluation by two independent reviewers. A numeric score was assigned as described previously (8). Results were compared by using standard statistical methods (1).

A total of seven strains were tested by using the animal model system described previously (9). The results of these studies (Table 1) indicate that there was considerable variability in the histopathologic abnormalities detected after immunization with the various strains.

Although preliminary tests suggested that strains obtained from patients with IBD were more active than strains from non-IBD sources (two from patients with antibiotic-associated colitis and one from a healthy individual), it can be seen that the histologic response does not appear to be related to the source of *B. vulgatus*. There does not appear to be any correlation between the antibody titer and the severity of disease, since the titers of antibody to the homologous strains used for immunization were relatively consistent for each strain, despite the differences in the degree of intestinal inflammation, as measured with the histologic scoring system. The mean histologic scores for all strains except BV20-15 were significantly higher than those

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TABLE 1. Comparison of immune response to *B. vulgatus* strains

Strain (source)	No. of animals tested	Mean score[a]	Antibody titer[b]
BV26-21 (IBD)	8	7.12 ± 0.82	≥1:2,048
BV20-15 (IBD)	6	5.33 ± 1.88	≥1:2,048
BV435 (non-IBD)	8	7.12 ± 1.76	≥1:2,048
BV16-4 (non-IBD)	8	7.25 ± 1.08	≥1:2,048
BV10-9 (non-IBD)	7	7.16 ± 1.77	≥1:4,096
40G2-33 (Guinea pig)	22	7.09 ± 1.95	≥1:4,096
40G1-38 (Guinea pig)	8	6.43 ± 1.18	≥1:2,048
Control[c]	14	5.20 ± 1.47	≤1:4

[a] The mean histologic score was based on the scores of all animals in each group. The scoring is based on a 0 to 12 scale. A score of 0 indicates that no lesions were present, whereas a score of 12 indicates that ulcerations and other inflammatory changes, including epithelial thinning, polymorphonuclear leukocytes in the lamina propria, and crypt abscesses, were detected in the cecum, colon, and rectum.

[b] Titers of three animals in each group were determined by microagglutination procedures. The results are expressed as the highest dilution showing obvious agglutination. The three samples in each group showed the same titer.

[c] The control was a nonimmunized guinea pig given 5% degraded carrageenan ad libitum in drinking water. All *B. vulgatus* whole-cell preparations were tested in controls.

for the nonimmune control group by use of a Student's *t* test for statistical comparisons.

These data support our previous reports detailing the enhancement of the inflammatory response in this animal model system after immunization and feeding with *B. vulgatus* and carrageenan (4–6, 8). Although there is considerable variability among strains when tested in this model system, there does not appear to be any particular source distribution associated with this activity. We are currently evaluating a number of enzymatic and antigenic markers in these strains of *B. vulgatus* to determine which factor(s) is responsible for the differences in immune enhancement of the inflammatory response in this animal model system.

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