Carrageenan-Induced Intestinal Injury in the Rat—A Model for Inflammatory Bowel Disease*

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ABSTRACT

The cause of inflammatory bowel disease (IBD) remains unknown. In this report, an attempt is made to produce a suitable animal model for studying the pathobiology of IBD, especially its pathogenesis. Sprague-Dawley rats were divided into four groups of six (A, B, C, D). The experimental design involved prior parenteral sensitization of groups A and B by a 1.5 percent solution of lambda degraded carrageenan followed by oral administration of the same solution for 30 days to groups A and C. The animals were then sacrificed, and the small intestine was evaluated for injury. Oral carrageenan caused significant intestinal injury as evidenced by ulceration, abnormal villous pattern, degree and extent of inflammation \( [p = 0.0001 \text{ for groups (A + C) versus (B + D)}] \). Prior sensitization aggravated the effects of oral carrageenan. Overall, the inflammation produced was reminiscent of human IBD in that there was pin-point ulceration, focality of lesions and lymphoid hyperplasia with microgranulomas. It was concluded that this carrageenan model may prove to be particularly useful for studying the pathobiology of human IBD.

Introduction

Idiopathic inflammatory bowel disease (IBD) is a chronic disease process comprising ulcerative colitis and Crohn's disease. Many factors have been implicated in its etiology including microbiologic agents, immunologic factors, ischemia, genetic factors, psychosomatic factors and diet.\(^3\,4\,5\,6\,8\,9\,10\,11\,12\) However, to date, the cause of IBD remains unknown, and treatment is still largely empirical.\(^9\,11\) One of the major setbacks to this type of research has been the fact that IBD, as it occurs in humans, has not
as yet been described in animals.\textsuperscript{2,3,13,21} The purpose of this study was to attempt to produce a suitable animal model for studying the pathobiology of IBD, especially the early stages pertaining to the pathogenesis of this disorder.

Carrageenan is a galactose polymer that is a well-known experimental inflammatory agent when administered parenterally.\textsuperscript{2,7,14,25} There are conflicting reports in the literature about the effects of orally administered carrageenan on the gastrointestinal tract of laboratory animals, with some reports denying that carrageenan has any effect on the gut.\textsuperscript{1,16,18,20,24} However, recent work on cell monolayers has shown that carrageenan has reproducible injurious effects on intestinal epithelial cells.\textsuperscript{15} In view of this, as well as the renewed interest in dietary factors with respect to the etiology of IBD,\textsuperscript{10} the present authors set out to investigate the possible effects of carrageenan on the intestinal tract of rats using a method that involves dietary and immunologic manipulation.

**Material and Methods**

Twenty-four female Sprague-Dawley rats\textsuperscript{*} each weighing 100 to 150 g, were divided into four groups of six (A, B, C, D). They were housed under standard conditions and fed standard rodent laboratory chow.\textsuperscript{†} On day 1, rats in groups A and B were each given a subcutaneous injection of a mixture of lambda degraded carrageenan and Freund's complete adjuvant;\textsuperscript{‡} in order to sensitize them (see experimental design, table I). On day 8, rats in groups A and C were given a 1.5 percent solution of lambda degraded carrageenan in their drinking water, whereas rats in groups B and D continued with ordinary drinking water. The bottles containing the water or carrageenan solutions were emptied, washed and replenished every day.

On day 38, a laparotomy was performed under ether anesthesia. The small bowel and its mesentery were examined in-situ, and the animals were then sacrificed by cervical dislocation. The small bowel and mesentery were removed and promptly immersed in a large shallow dish containing 10 percent neutral buffered formaldehyde. A 20 cm long segment of small bowel (the 40 to 60 cm segment distal to the pylorus) was removed and opened longitudinally along its antimesenteric border. By a gentle motion under formaldehyde, this segment was cleared of intestinal contents leaving a mucosa that was suitable for examination with a dissecting microscope; mucosal examination was carried out using ×10 magnification. Attention was then turned towards the rest of the small bowel. The intestinal contents were flushed out with formaldehyde, and the specimen was left in the container for definitive fixation in formaldehyde. Based on the gross appearance of the lesions as well as their morphology on the dissecting microscope, 10 representative tissue blocks from both the intact tubular segments and the opened portion of bowel were submitted for light microscopy; the representative tissue blocks also included some normal-appearing areas. The sites of previous

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<td><strong>Experimental Design</strong></td>
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<tr>
<td>Day 1</td>
</tr>
<tr>
<td>Day 8</td>
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<td>Day 38</td>
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SC = subcutaneous \ CG = carrageenan

\textsuperscript{*} Charles River Canada, Quebec, Canada.
\textsuperscript{†} Purina Mills Inc., St. Louis, MO.
\textsuperscript{‡} Sigma Chemical Co., St. Louis, MO.
subcutaneous injection in groups A and B were exposed and sampled for microscopy.

Results

Gross external examination of the small bowel in all four groups showed variably-sized Peyer's patches. However, the Peyer's patches were more prominent in groups A and C than in groups B and D, as were the mesenteric lymph nodes (figure 1). Examination of the bowel mucosa through the dissecting microscope revealed a generally regular finger- or leaf-like villous architecture in groups B and D, even in those areas that overlaid Peyer's patches. Focal areas depicting partial obliteration or total effacement of the villous architecture were seen mostly in groups A and C (figure 2). Some of these abnormal areas overlaid Peyer's patches whereas others were not associated with Peyer's patches.

Ten different small bowel sections were evaluated for possible histopathologic changes using a number of indices of inflammation (table II). For each of the four groups (A, B, C, D), the mean grade or score for each of the indices of inflammation was calculated. The results are shown in the bar graphs in figure 3. In order to compare the four groups (A, B, C, D), a set of orthogonal contrasts amongst the mean grades (figure 3) of the inflammatory indices was constructed. Each of these contrasts was tested by an F test with one and 20 degrees of freedom, and the results are shown at the bottom in figure 3.

The histopathologic changes generally correlated with the gross morphology in that there was marked lymphoid hyperplasia of the Peyer's patches, with corresponding attenuation of the overlying mucosa and underlying muscularis propria (figure 4). The hyperplastic lymphoid follicles were associated with marked expansion of the interfollicular lymphoid tissue. A few granulomas that were not of foreign body type were seen within some of the Peyer's patches. Sec-
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TABLE II
Indices Of Inflammation

<table>
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<tr>
<th>GRADE</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Ulceration</td>
<td>Nil</td>
<td>Few; Pin-point</td>
<td>Many; Pin-point</td>
<td>Many; Sizable</td>
</tr>
<tr>
<td>Abnormal Villous Pattern</td>
<td>Normal</td>
<td>Few</td>
<td>Many</td>
<td>Extensive</td>
</tr>
<tr>
<td>Degree of Inflammation</td>
<td>Normal</td>
<td>Slight</td>
<td>Moderate</td>
<td>Marked</td>
</tr>
<tr>
<td>Extent of Inflammation</td>
<td>Nil</td>
<td>Involvement of villi only</td>
<td>Whole mucosa</td>
<td>Beyond mucosa</td>
</tr>
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</table>

Histopathology also correlated with the villous architectural abnormalities as seen through the dissecting microscope. These abnormalities in villous pattern included villous shortening, bulbous forms, variable degrees of fusion, and total villous effacement (figure 5). These changes were most marked in group A (A > C at p = 0.0015). Although groups B and D did show a few shortened and partially fused villi configurations, the changes in groups A and C were much more pronounced (A + C > B + D at p = 0.0001).

Areas of mucosal ulceration, which were mostly of small size (pin-point ulceration), were commonly seen in group A (figure 6), with fewer areas in group C (A > C at p = 0.03). The areas of ulceration were often associated with mucosal congestion and edema. Groups B and D hardly showed any areas of ulceration (A + C > B + D at p = 0.0001). The inflammatory cell infiltrate was marked in groups A and C, and consisted predominantly of lymphocytes.

![Figure 3](image-url)
Figure 4. Cross-section of small intestine showing hyperplastic lymphoid follicles (arrows) and marked expansion of the interfollicular lymphoid tissue. Hematoxylin and eosin ×16.5.

plasma cells and histiocytes; polymorphs were present in lesser numbers (figure 6). The inflammatory changes appeared to be focal in that some sections from a given case were virtually normal, whereas others from the same case showed the above abnormalities. These inflammatory changes were accompanied by other features that were less easy to quantify, such as increased epithelial mitotic activity, hyperplasia of the crypt epithelium, and some reparative changes in the mucosa and submucosa. Groups B and D generally showed only the usual compliment of lymphocytes and plasma cells in the lamina propria. With respect to the extent of the inflammatory process, the focal areas of inflammation in groups A and C specimens often extended beyond the mucosa to involve the submucosa whereas in groups B and D, there were hardly any inflammatory cells beyond the confines of the mucosa or Peyer’s patches.

Thus, in summary, the administration of oral carrageenan (groups A and C) produced significant bowel lesions as evaluated by the indices of inflammation shown in table II, and prior sensitization of the animals using a subcutaneous injection (group A versus C) aggravated the lesions.

Discussion

The etiology of IBD is unknown. Without knowledge about the pathogenesis of this disease, it is difficult to pro-
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Figure 6. High-power view of mucosa depicting areas of pin-point ulceration (arrow-heads); note the intraepithelial inflammatory cell exudate (arrows). Hematoxylin and eosin × 300.

vide preventive or therapeutic measures based upon sound principles. This is reflected in the contemporary drug treatment of these patients which is largely empirical with sulfasalazine and corticosteroids being the mainstay of therapy. At the same time the limitations, owing largely to ethical considerations, of conducting investigative work pertaining to the pathogenesis of IBD on humans, are well-known. There are thus compelling reasons for designing a suitable animal model for studying the various aspects of the pathobiology of IBD. Indeed, many previous studies have sought to address this issue. The idea of an infectious etiology generated much interest but remains unproven to date. Spurred on by the morphologic abnormalities in gastrointestinal and mesenteric lymphatics and lymphoid tissue, some investigators explored the idea of experimental manipulation of the lymphatics in an attempt to induce IBD, but this line of research does not seem to have provided most of the answers. Similarly, other avenues of research variously implicating ischemia, topical chemotactic peptides and genetic factors have had their shortcomings.

The idea of a dietary factor being implicated in the pathogenesis of IBD has been around for a long time. The present authors were interested in exploring further this avenue of experimentation because of its natural appeal; that is, to the extent that oral administration of a food substance is a physiologic process and does not involve invasive procedures, this closely simulates IBD as it occurs in humans in whom it seemingly arises de novo.

Our results are in accord with those of some of the previous investigators in that carrageenan alone produced inflammatory bowel lesions. However, these lesions were exacerbated by prior subcutaneous sensitization, reminiscent of what was shown by Matsumoto et al. To the extent that there was demonstrated pin-point ulceration, villous architectural distortion, focality of lesions, and lymphoid hyperplasia with microgranulomas, the experimental bowel lesions herein described are similar to the early stages of IBD as it occurs in humans. These findings lend some support to the notion that a dietary factor may be involved in the pathogenesis of human IBD. Indeed, it is known that the incidence of IBD increased sharply in the last 50 years, that IBD is more common in some parts of the world, and that its incidence changes with human migration; this epidemiologic profile would be compatible with an environmental factor such as diet. The aggravation of the
bowel lesions by prior parenteral sensitization in this study suggests that immunologic factors may play a role in the exacerbation or relapse of IBD.

In conclusion, this study provides a suitable animal model for investigating the pathobiology of experimental inflammatory bowel disease, and it may prove to be particularly useful for studying the cellular mechanisms involved in the pathogenesis thereof. There is a real possibility that these findings may be of relevance to the pathobiology of human IBD.

Acknowledgments

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References