Chemotactic Activity in Inflammatory Bowel Disease
Role of Leukotriene B₄

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An important histologic feature of inflammatory bowel disease (IBD) is infiltration of the colonic mucosa with neutrophils. To investigate the nature of the chemotactic agents responsible for this infiltration, colonic mucosa from three normals and nine patients with inflammatory bowel disease (seven ulcerative colitis, two Crohn's colitis) was assayed for chemotactic activity for human neutrophils in vitro in a Boyden chamber. There was more (>10-fold more) chemotactic activity in homogenates of inflammatory bowel disease mucosa than in homogenates of normal colonic mucosa. Analysis of the chemotactic activity in the inflammatory bowel disease mucosa revealed that most was lipid extractable. Moreover, when the lipid extract was fractionated by reverse-phase high-pressure liquid chromatography, the only fraction with significant chemotactic activity was the fraction that coeluted with leukotriene B₄. The chemotactic response to IBD mucosa was blocked by anti-LTB₄ antisera. The amount of chemotactic activity in lipid extracts of different inflammatory bowel disease specimens correlated well with the concentration of leukotriene B₄ measured by UV absorbance (250 ng/g of mucosa). These data suggest that leukotriene B₄ is an important stimulus to neutrophil chemotaxis in inflammatory bowel disease and, thus, may play a major role in the amplification of the inflammatory response in this condition.

KEY WORDS: inflammatory bowel disease; Crohn's disease; ulcerative colitis; chemotaxis; neutrophil.

Ulcerative colitis and Crohn's disease are inflammatory diseases of unknown etiology. Not only is the etiology unknown, but the roles of different soluble mediators of inflammation in the amplification of the inflammatory response in these diseases are largely undefined. There has been some recent interest in the potential role of arachidonate metabolites as mediators in the pathogenesis of inflammation in inflammatory bowel disease (IBD) (1–3). We reported high concentrations of one soluble mediator of inflammation, leukotriene B₄ (LTB₄), in the colonic mucosa of patients with IBD (4). LTB₄ is a potent chemotactic agent for human neutrophils and increases vascular permeability (5, 6). Tissue infiltration with neutrophils and mucosal edema are prominent histologic features of IBD. In both Crohn's disease and ulcerative colitis there is a great flux of neutrophils out of the circulation, into the inflamed mucosa, and then out into the intesti-
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Tional lumen (7, 8). This flux of neutrophils can be quantitated by giving the patient 111In-labeled neutrophils and measuring the appearance of radioactivity in the gut.

To define, in part, the contributions of different inflammatory mediators to the inflammatory response in IBD, we have attempted to quantitate and chemically characterize neutrophil chemotactic activity in IBD. Our studies demonstrate that there is much more chemotactic activity in IBD mucosa than normal mucosa. Moreover, chemical characterization of this chemotactic activity reveals that it is lipid extractable and cochromatographs with LTB4 on reverse-phase high-pressure liquid chromatography (HPLC).

MATERIALS AND METHODS

Patients. Inflamed IBD mucosa was obtained from seven patients undergoing colectomy for ulcerative colitis and two undergoing colectomy for Crohn's colitis. There were six men and three women whose ages ranged from 17 to 61 years. The duration of illness was 2–40 years. At the time of operation, seven patients were receiving corticosteroids, four were receiving sulfasalazine, and seven patients were receiving total parenteral nutrition. The diagnoses were made on the basis of clinical and pathologic characteristics. All had clinically and histologically active disease at the time of operation. Pathologic studies of each of the operative specimens from the patients with IBD revealed ulcers and neutrophil infiltration. Three samples of normal mucosa came from the uninvolved areas of colonic resections for adenocarcinoma (4). The surgical specimens, both from patients with IBD and from patients with adenocarcinoma were reviewed by a pathologist immediately after operation. The pathologist released only those portions of the specimens not required for pathologic analysis. In some cases, the amount of colonic tissue released was inadequate for the performance of all studies. Thus, not all of the studies presented were performed using mucosa from all of the patients. This study was approved by the Human Studies Committee of the Jewish Hospital of St. Louis (approval granted June 25, 1982).

Chemotaxis Assay. The chemotaxis assay used is a modification of that of Gallin et al (9). Purified human neutrophils were prepared from heparinized blood by dextran sedimentation and centrifugation over a Ficoll-Hypaque gradient, followed by a 140g centrifugation to remove platelets and hypotonic lysis of erythrocytes (10). Two hundred forty million neutrophils were incubated at 37°C for 45 min with 4μCi of 31Cr (109 mCi/mg) (ICN Radiochemicals, Irvine, California) in 6 ml of a balanced salt medium buffered at pH 7.4. After incorporation of 31Cr, the neutrophils were washed three times and resuspended in Eagle's minimum essential medium (MEM) with 1% human serum albumin (HSA) at 8 x 106 cells/ml. The chemotactic agent was solubilized in the same medium and 0.24 ml was added to the bottom of a blind-well chamber. Two 13-mm-diameter 5.0-μm pore filters (Millipore, Bedford, Massachusetts) separated the chemotactic agent from 0.4 ml of the neutrophil suspension added to the top chamber. After a 3-hr incubation at 37°C in a 5% CO2 incubator, the top chamber was emptied and the two filters were separated and counted in a gamma counter. Radioactivity in the fluid in the upper and lower chambers was also assayed but effectively all of the radioactivity was in the filters. The percentage of total cpm found in the lower filter reflects the number of neutrophils that had migrated through the upper filter and entered the lower filter. This is a reflection of the chemotactic activity of the contents of the lower chamber. The cpm in the lower filter were expressed as percent of total cpm in lower and upper filter and compared to a control with only medium in the bottom chamber. All assays were done in triplicate.

Chemotaxis Studies with Antisera. Either rabbit anti-LTB4 antiserum or normal rabbit serum was added to the chemoattractant (LTB4, mucosal homogenate, or mucosal extract) in the bottom chamber of the modified Boyden chamber prior to the chemotaxis assay. The anti-LTB4 antiserum was a generous gift of E. Hayes, Merck, Rahway, New Jersey. In these experiments, the response to the chemoattractant in the absence of antiserum was normalized to 100%. The chemotactic response in the presence of antiserum was expressed as a percentage of the response in the absence of antiserum.

Preparation of Samples for Chemotaxis Assay. To prepare the homogenate, colonic mucosa was scraped from surgical specimens with a scalpel. Aliquots (200–500 mg) of the scraped mucosa were homogenized by hand in a Dounce homogenizer in 1–2.5 ml distilled water while on ice. The homogenate was mixed with an equal volume of 2X MEM-1% HSA and diluted to the desired concentration with medium for the assay. For certain chemotaxis assays the tissue was not homogenized but rather the mucosa was incubated at 37°C in MEM with 1 mg/ml BSA for 30 min and then the media was removed and the chemotactic activity in the media assayed.

For the lipid extract, after homogenization, the mucosal suspension was brought to 80 mg/ml with distilled water. Lipids were extracted with 2.5 ml of the chloroform–methanol–5% formic acid (1:2:1:2:0.1) per milliliter of homogenate (4). The chloroform layer was removed and another 1.2 ml of chloroform added. The combined chloroform layers were dried under nitrogen and solubilized at the desired concentration in MEM–1% HSA for the chemotaxis assay. For certain extractions the tissue was placed in 3.8 ml of water–methanol–chloroform (0.8:2:1) for 30 min on ice prior to homogenization.

For the HPLC fractions, the lipid extract was fractionated by HPLC on an Altex 4.6 x 250-mm UltraspHERE ODS reverse-phase column eluted isocratically with methanol–water–acetic acid (75:25:0.01) at 1 ml/min (11). The desired amount of lipid extract was dried under nitrogen, dissolved in 0.1 ml of the eluting mixture, and injected into the column. UV absorbance was monitored at 270 nm for 20 min and then at 235 nm. The 3-ml fraction containing LTB4 was collected separately; the remainder of the eluate was collected in six 9-ml fractions. The