CYTOKINES INCREASE PROLIFERATION OF HUMAN INTESTINAL SMOOTH MUSCLE CELLS: POSSIBLE ROLE IN INFLAMMATION-INDUCED STRICTURE FORMATION

MICHAEL W. OWENS and MATTHEW B. GRISHAM

Departments of Medicine, and Physiology and Biophysics
Overton Brooks Veterans Affairs and Louisiana State University Medical Center
Shreveport, Louisiana 71130

Abstract—Crohn’s disease is an idiopathic, chronic inflammation of the gastrointestinal tract that causes narrowing and stricturing of primarily the small and large intestine. Although the mechanism(s) by which chronic inflammation promotes stricture formation remain to be defined, it does appear to be associated histologically with a hyperplasia of smooth muscle cells and an increased deposition of collagen within the bowel wall. The objective of this study was to assess the effect of two proinflammatory cytokines, tumor necrosis factor and interleukin-1, on the proliferation of human intestinal smooth muscle cells in vitro. Human intestinal smooth muscle cells were seeded at subconfluent densities into 24-well plates in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. Human recombinant tumor necrosis factor (0.1–100 ng/ml), interleukin-1 (0.1–500 ng/ml), or control medium (without cytokines) was then added to the cells and incubation continued for 48 or 72 h. Proliferation was determined by the incorporation of tritiated thymidine, added during the final 18 h, into the cellular DNA of the smooth muscle cells. Both cytokines caused a significant dose-dependent increase in intestinal smooth muscle cell proliferation relative to control. These results suggest that the interleukin-1 and tumor necrosis factor produced during chronic inflammation in vivo may enhance the proliferation of smooth muscle cells within the intestinal bowel wall and hence potentially contribute to the narrowing and stricturing of the intestine that is observed in Crohn’s disease.

INTRODUCTION

Crohn’s disease (CD) is an idiopathic, chronic inflammation of the gastrointestinal tract that affects primarily the small and large intestine. The inflammation

1This work was supported by a Merit Review Grant from the Veterans Affairs Medical Research Service (M.W.O.) and by a grant from the NIH (DK43785, Project 6; M.B.G.).

0360-3997/93/0800-0481$7.00/0 © 1993 Plenum Publishing Corporation
is transmural in nature and is characterized by the infiltration of large numbers of leukocytes (e.g., neutrophils, macrophages, eosinophils, and lymphocytes) into the mucosal interstitium (1). A common feature of CD is a narrowing or stricturing of the bowel lumen. Histopathological specimens obtained from patients with active disease reveal dramatic thickening of the bowel wall with increased numbers of smooth muscle cells (2). In addition to this smooth muscle hyperplasia, Graham and coworkers have demonstrated that there is a dramatic increase in the deposition of collagen type V within the bowel wall, which appears to be synthesized by intestinal smooth muscle cells (3).

Stricture formation is a clinically important complication of CD because of its adverse effects on intestinal structure and function (e.g., absorption and motility) (4–6). Furthermore, the bowel lumen may become obstructed if the inflammation and stricturing are severe enough. In many cases, surgical intervention in the form of bowel resection is required. Although the mechanisms by which chronic inflammation promotes stricture formation in the gastrointestinal tract have not been entirely defined, there is some evidence to suggest that proinflammatory mediators released by leukocytes and parachymal cells may increase smooth muscle proliferation as well as enhance collagen synthesis and deposition by these cells (7). Therefore, the objective of this study was to assess the ability of two cytokines known to be elevated in experimental and/or human inflammatory bowel disease (IBD) to enhance proliferation of human intestinal smooth muscle cells in vitro.

MATERIALS AND METHODS

Materials. Human recombinant tumor necrosis factor alpha (TNF) (10⁷ units/mg protein) was obtained from Amgen. Interleukin-1-β (IL-1β) (5 x 10⁷ units/mg protein) was obtained from Sigma Chemical Co. (St. Louis, Missouri). [³H]Thymidine (specific activity 6.7 Ci/mmol) was obtained from New England Nuclear. NuSerum was purchased from Collaborative Research. Dulbecco’s modified Eagle’s medium (DMEM) and fetal bovine serum were obtained from Gibco. All chemical reagents were purchased from Sigma.

Human Intestinal Smooth Muscle Cells. A human intestinal smooth muscle cell (HISMC) line (No. 1692) was obtained from the American Type Culture Collection (Rockville, Maryland). The cells were grown in DMEM supplemented with 10% NuSerum, glutamine (2 mM), and gentamicin (50 μg/ml) (subsequently referred to as a complete medium) in a 95% air–5% CO₂, humidified atmosphere. Confluent monolayers were passaged by trypsinization. Passages 2–10 were used for these experiments.

HISMC Proliferation Assay. The effect of TNF and IL-1β on HISMC proliferation was assessed by measuring the incorporation of [³H]thymidine into the cellular DNA of subconfluent monolayers. HISMC were grown to confluence in tissue culture flasks in complete medium. Cells were then washed with phosphate-buffered saline (PBS), exposed to trypsin (0.25%), and suspended (1 x 10⁵ cells/ml) in DMEM with 10% heat-inactivated fetal bovine serum (FBS), glutamine (2 mM), and gentamicin (50 μg/ml). The HISMC were then plated at a density of 5 x 10⁴ cells/well for the 48-h experiments and 3 x 10⁴ cells/well for the 72-h experiments in quadruplicate wells in flat-bottom 24-well plates (Gibco) and incubated at 37°C for 20 h in 95% air–5% CO₂. TNF (0.1-