SUPPLEMENTAL METHODS

RNA extraction and mRNA expression

Total RNA was extracted from the isolated intestinal muscularis externae of mice at a series of specific time points after intestinal manipulation (0, 3, 6, 12 and 24 hours, N=5 each). The isolated muscularis was immediately snap frozen in liquid nitrogen and stored at -80°C. Total RNA extraction was performed as previously described [22], using the guanidinium thiocyanate phenol-chloroform extraction method. Standard SYBR Green two-step, real-time RT-PCR using GAPDH as the endogenous reference was performed. Primer sequences for mouse Egr-1 were the following [sense: 5'-GTCCTTTTCTGACATCGCTCTGA 3'] [anti-sense: 3'-CGAGTCGTTTGGCTGGGATA-5']. Primer sequences for mouse GAPDH were the following [sense: 5'-TGA AGG TCG GTG TGA ACG GAT TTG GC-3'] [antisense: 5'-CAT GTA GGC CAT GAG GTC CAC CAC-3']. Quantification of mRNA expression was normalized to the GAPDH reference gene and calculated relative to control using the comparative CT method. Gel electrophoresis was performed for the primers to confirm the absence of non-specific bands and that the amplicons were of the expected size. Melting curve analysis was performed for each PCR reaction to ensure amplification of a single product.

Western Blot

The isolated jejunal muscularis externa was dissected from mouse small intestine at 4°C and immediately flash frozen in liquid nitrogen. Tissue was homogenized on ice in a SDS/Tris-HCl lysis buffer containing protease inhibitors. A protein assay was performed on the samples using the BCA Protein Assay Kit (Pierce, Rockville, IL). Protein concentrations were equilibrated, and a gel loading buffer containing Tris-HCl, SDS, glycerol, bromophenol blue, and β-mercaptoethanol was added to each sample. Approximately 40µg of protein per sample was loaded onto 7.5% precast gels (Bio-Rad, Hercules, CA) alongside a positive control (NIH/3T3 whole cell lysate from Santa Cruz Biotechnology, Santa Cruz, CA) and a molecular weight marker (Kaleidoscope from Bio-Rad). Gels were run on a Bio-Rad Mini-Protean 3 Electrophoresis Cell Module and transferred to a Hybond-P protean transfer membrane (Amersham, Piscataway, NJ) with the Bio-Rad Mini Trans Blot Module. Membranes were blocked in TBS/T with 5% NFDM and incubated overnight in a 1:200 dilution of primary antibody (diluted in blocking buffer). Both the Egr-1 (C-19) and actin (C-11) antibodies were purchased from Santa Cruz Biotechnology. Secondary antibodies (Pierce, Rockville, IL) were diluted in blocking buffer and applied for one hour. SuperSignal West Dura (for Egr-1) and SuperSignal West Pico (for Actin) chemiluminescent substrates were applied to membranes for ten minutes. Membranes were then developed onto Kodak BioMax Light Film. Images were scanned and densitometry was performed using Quantity One.

Histo- and immunohistochemistry stainings

Whole-mounts of the jejunal intestinal muscularis were investigated for the presence of resident and recruited myeloperoxidase-positive neutrophils and monocytes, as well as, the cellular localization of Egr-1 protein as described previously[15]. To visualize polymorphonuclear neutrophils, freshly prepared whole-mounts were subjected to a myeloperoxidase stain (10mg Hanker-Yates reagent (Sigma, St. Louis, MO), 10mL KRB and 100µL 3% hydrogen peroxide) for 20 min at room temperature. The reaction was stopped by washing the whole-mounts in cold KRB.

Muscularis whole-mounts were also processed for immunohistochemistry to detect resident and recruited macrophages/monocytes. Whole-mounts were blocked in 10% normal horse serum diluted in PBS (phosphate-buffered hypertonic saline, 1.8% NaCl in 0.01 M phosphate buffer, pH 7.4) containing 0.1% Triton X-100 for 1 hour at room temperature before incubation in primary antibody. We used a Serotec F4/80 antibody conjugated to alexa-488 (1:200, rat polyclonal anti-mouse antibody, Harlan Bioproducts, Indianapolis, Ind.) to visualize macrophages/monocytes and an Egr-1 antibody (1:200, C-19 rabbit polyclonal anti-human antibody, Santa Cruz Biotechnology, Santa Cruz, CA). All primary antibodies were diluted in antibody diluent. Whole-mounts were incubated in primary antibody at room temperature overnight. The specimens were then incubated in the appropriate secondary antibody (1:10,000, Cy3 donkey-anti-rabbit or 1:1000 Cy5 donkey-anit-rabbit secondary antibodies, Jackson Immunoresearch, West Grove, PA) at room temperature for 2 hours followed by three changes in PBS for 5 min each. Whole-mounts were cover slipped and inspected by light or fluorescent microscopy (Leica DMRX, W. Nushbaum, Inc., McHenry, IL). Leukocytes were counted in 5 randomly chosen areas in each specimen at a magnification of 200X. Nonspecific isotype-matched antibodies and primary antibody incubation without secondary antibody were used as negative controls.

Measurement of nitric oxide and prostaglandins in muscularis cultured supernatant

The release of nitric oxide (NO) and prostaglandins was measured in culture media supernatant of the isolated muscularis externa obtained from Egr-$1^{+/+}$ and Egr-$1^{-/-}$ animals 24 hours after intestinal manipulation and from control unoperated genetically matched animals.

The release of NO into the tissue culture supernatant over a period of 24 hours was assayed by a standard Griess reaction adapted to microplates, as described previously[23]. Nitrite was quantitated using $NaNO_2$ as a standard and results were expressed as μM nitrite/g tissue. Culture supernatants were assayed for the measurement of prostaglandin secretion by enzyme immunoassay (Cayman Chemicals, Ann Arbor, MI). The ELISA was carried out in a 1:10 and 1:30 dilution. The assay was corrected for wet tissue weights and the prostaglandin ELISA kit sensitivity was 20 pg/ml.