BASIC–ALIMENTARY TRACT

Mechanisms Underlying the Maintenance of Muscle Hypercontractility in a Model of Postinfective Gut Dysfunction

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Background & Aims: **Acute gastroenteritis is a strong risk factor for the development of irritable bowel syndrome (IBS). We have developed an animal model in which transient acute infection leads to persistent muscle hypercontractility. Here, we investigate the mechanisms underlying the maintenance of this hypercontractility in the postinfective (PI) state.** ***Methods:*** **Muscle contraction and messenger RNA (mRNA) or protein expression of cytokines were examined from jejunal longitudinal muscle cells of NIH Swiss mice infected with *Trichinella spiralis* or incubated with or without cytokines.** ***Results:*** **During acute infection, interleukin (IL)-4 or IL-13, transforming growth factor (TGF)-β1, and cyclooxygenase (COX)-2 were increased in the muscle layer ($P < .05$). In the PI phase of the model, T helper (Th)2 cytokines returned to normal, but TGF-β1 remained in the muscle ($P < .05$). Exposure of muscle cells to IL-4 or IL-13 increased TGF-β1 ($P < .01$), COX-2 protein, and prostaglandin (PG)E_2. Exposure of muscle cells to TGF-β1 increased PGE_2 ($P < .05$) and COX-2 protein. Incubation of tissue with IL-4, IL-13, TGF-β1, or PGE_2 enhanced carbachol-induced muscle cell contractility ($P < .05$). COX-2 inhibitor attenuated TGF-β1–induced muscle hypercontractility ($P < .05$).** ***Conclusions:*** **These results support the hypothesis that Th2 cytokines induce muscle hypercontractility during infection by a direct action on smooth muscle. The maintenance of hypercontractility results from Th2 cytokine-induced expression of TGF-β1 and the subsequent up-regulation of COX-2 and PGE_2 at the level of the smooth muscle cell. We propose that PI gut dysfunction reflects mediator production in the neuromuscular tissues and that this may occur in PI-IBS.**

Irritable bowel syndrome (IBS) is the most common disorder diagnosed by gastroenterologists. Acute gastroenteritis is a strong risk factor for the IBS.[1] However, mechanisms underlying this association are not understood. Studies have shown increased numbers of lymphocytes in the colon of patients with postinfective (PI) IBS (PI-IBS),[2,3] suggesting immune activation. However, the mechanism whereby immune activation in the mucosal compartment leads to long-lasting changes in the physiology of the deeper neuromuscular tissue is poorly understood.

We have developed an animal model to study how transient enteric infection leads to long-term gut dysfunction. Using intestinal muscle contractility as an in vitro marker of gut dysfunction, we have shown that primary infection of NIH Swiss mice by the nematode parasite *Trichinella spiralis* results in the hypercontractility of intestinal muscle that persists for 6 weeks after recovery from infection when there is no discernable mucosal inflammation.[4,5] Because hypercontractility did occur in infected athymic[6] or signal transducer and activator of transcription (STAT)-6-deficient mice,[7] we concluded that T helper (Th)2 cytokines interleukin (IL)-4 and IL-13, but not IL-9,[8] were critical for the induction of muscle hypercontractility. Subsequent studies showed that activation of cyclooxygenase (COX)-2 in the muscularis externa was necessary for the persistence of muscle hypercontractility postinfection.[5]

The present study was undertaken to explore mechanisms that link the induction and the persistence of muscle dysfunction in this model. IL-4 exerts its biologic effects by binding to the IL-4 receptor (R) α-chain, a component of both the type 1 IL-4R and the type 2 IL-4R.[9–11] In the type 2 IL-4R, IL-4Rα is paired with IL-13Rα1, to which IL-13 also binds.[12,13] We have

Abbreviations used in this paper: COX, cyclooxygenase; IFN, interferon; IL, interleukin; JAK, janus kinase; LMMP, longitudinal muscle layer myenteric plexus; PG, prostaglandin; PI, postinfective; R, receptor; STAT, signal transducer and activator of transcription; Th, T helper; TGF, transforming growth factor.

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shown that IL-4 or IL-13 enhances murine jejunal muscle contractility.[14] TGF-β exerts its multiple actions through 2 types of transmembrane receptors (type I and type II).[15] There are 3 subtypes of TGF-β: TGF-β1, TGF-β2, and TGF-β3. TGF-β1 has been studied mostly in terms of the biologic effects and receptor bindings. The TGF-β type II receptor can bind TGF-β1 by itself and requires the type IR for signal transduction[16] but not for ligand binding.[17] TGF-β1 is known to stimulate COX-2 expression and PGE_2 release in human airway smooth muscle cells.[18] And TGF-β1–induced enhancement of vasoconstrictor responses is also reported.[19]

Thus, we have investigated whether muscle hypercontractility at the PI state is maintained at the level of smooth muscle cell and examined the roles and sites of action of Th2 cytokines, TGF-β1, and COX-2–induced PGE_2 production in the maintenance of muscle hypercontractility. Our results describe a model in which Th2 cytokines initiate muscle hypercontractility during the acute infection but in which the changes are maintained postinfection by the presence of TGF-β1 in the muscularis externa. TGF-β1 induces COX-2 and promotes PGE_2 production at the level of the muscle cell, thus maintaining hypercontractility.

Materials and Methods

Materials

The following materials were used in this study: collagenase (CLS type l), trypsin inhibitor, BSA, phenylmethylsulfonyl fluoride (PMSF), aprotinin, leupeptine, nonidet P-40, acrolein, PGE_2, peroxidase conjugated-anti-goat/sheep IgG from Sigma Chemical (St. Louis, MO); HEPES from Bioshop Canada (Burlington, ON, Canada); IL-4, IL-13, TGF-β1, TGF-β1 immunoassay kit from R&D Systems (Minneapolis, MN); DMEM, antibiotic-antimycotic from Gibco BRL Life Technologies (Gaithersburg, MD); PGE_2 assay kit from BIOTRAK (Oakville, ON, Canada); COX-2 (murine) polyclonal antibody from Santa Cruz Biotechnology (Santa Cruz, CA); *DC* protein assay reagent, amplified opti-4CN substrate kit from Bio-Rad (Hercules, CA); PVDF membrane from Amersham Pharmacia Biotech (Piscataway, NJ); Celecoxib from Pfizer (New York, NY).

Mice

Studies were performed on male NIH Swiss mice (purchased from the National Cancer Institute, Frederick, MD), with or without *T spiralis* infection, between 6 and 10 weeks of age. Mice were kept in filter-isolated cages in groups of 4 or 5 in positive-pressure rooms with a constant ambient temperature and a 14:10-hour, light/dark cycle. All experiments were approved by the Animal Care Committee at McMaster University and were conducted in accordance with the guidelines of the Canadian Council on Animal Care.

Trichinella Infection

Mice were infected by administration of 0.1 mL phosphate-buffered saline containing 375 *T spiralis* larvae by gavage. The larvae were obtained from infected rodents 60–90 days after infection using a modification of the technique described by Castro and Fairbairn.[20] The *T spiralis* culture originated in the department of Zoology at the University of Toronto, and the colony was maintained through serial infections alternating between male Sprague-Dawley rats and male CD1 mice.

Preparation of Dispersed Smooth Muscle Cells

Muscle cells were isolated from longitudinal muscle layer myenteric plexus (LMMP) of the NIH Swiss mice jejunum by a method similar to that used by Bitar and Makhlouf[21] to prepare smooth muscle cells from the guinea pig stomach. The uninfected mice and the infected mice with *T spiralis* were killed by cervical dislocation. The jejunum was removed and placed in DMEM with 1% antibiotic-antimycotic. LMMP was peeled off from jejunum. The LMMP was preincubated with or without cytokines (10–100 ng/mL IL-4, 10–100 ng/mL IL-13, 1–100 ng/mL TGF-β1) or 0.1 nmol/L PGE_2 for 16 hours or preincubated with COX-2 inhibitor, 10^{-5} mol/L celecoxib for 2 hours in the 5% CO_2 incubator. The LMMP was incubated for 2 successive 10-minute periods at 31°C in 10 mL HEPES medium (98.1 mmol/L NaCl, 3.87 mmol/L KCl, 2.42 mmol/L $NaH_2PO_4H_2O$, 4.86 mmol/L L-glutamic-acid, 4.86 mmol/L fumaric acid, 4.86 mmol/L pyruvate, 11.17 mmol/L glucose, 1.79 mmol/L $CaCl_2$, 1.2 mmol/L $MgSO_47H_2O$, 23.5 mmol/L HEPES, pH 7.4) containing 1 mg/mL of collagenase, BSA, and trypsin inhibitor. After incubation, the partly digested LMMP was washed with enzyme-free HEPES medium and reincubated in 10 mL fresh HEPES medium to allow the cells to disperse spontaneously. Cells were then harvested by filtration through a 210-μm polyester mesh.

Reverse-Transcription Polymerase Chain Reaction for TGF-β1, IL-4R, and TGF-β Type IIR

Expression of mRNA of TGF-β1 in LMMP with or without *T spiralis* infection and IL-4R and TGF-β type IIR in cultured jejunal smooth muscle cells was investigated by a method described previously.[22] Total cellular RNA was isolated based on previously described guanidium isothiocyanate method.[23] The concentration of RNA was determined by measuring absorbance at 260 nm, and its purity was confirmed using the ratio of absorbency at 260 nm to that at 280 nm. RNA was stored at −70°C until used for reverse-transcription polymerase chain reaction (RT-PCR). mRNA was then reverse transcribed as described previously to yield cDNA, and the cDNA was amplified by PCR using gene-specific primers. 0.1 μg cDNA in 50-μL aliquots were then mixed with 20 pmol/L upstream and downstream primers, which were designed based on the available cDNA sequence: each of upstream 5′-TCA