Altered inflammatory gene expression underlies increased susceptibility to murine postoperative ileus with advancing age

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Moore BA, Albers KM, Davis BM, Grandis JR, Tögel S, Bauer AJ. Altered inflammatory gene expression underlies increased susceptibility to murine postoperative ileus with advancing age. Am J Physiol Gastrointest Liver Physiol 292: G1650–G1659, 2007. First published March 15, 2007; doi:10.1152/ajpgi.00570.2006.—Susceptibility to postoperative ileus following abdominal surgery increases with advancing age. The mechanisms underlying this phenomenon are unknown. This study compares functional and molecular endpoints between young-adult (2 mo old), middle-aged (15 mo old), and elderly mice (26–30 mo old) to identify potential mechanisms. Susceptibility to ileus was assessed by measuring gastrointestinal transit (geometric center) 24 h after anesthesia, laparotomy, and light manipulation (LM) of the small bowel. Proinflammatory (IL-6, COX-2, inducible nitric oxide synthase) and anti-inflammatory (IL-10, heme oxygenase-1) gene and protein expressions were determined by real time RT-PCR, Western blot, and ELISA. LM did not alter gastrointestinal transit in young animals (geometric center = 8.8 ± 0.9), but transit was increasingly delayed in middle-aged (6.9 ± 0.8, P = 0.03) and elderly animals (4.7 ± 0.6, P = 0.013). Despite the lack of LM effect on transit in young mice, IL-6 and COX-2 mRNA expressions were significantly increased postoperatively (165 ± 24-fold and 2.9 ± 0.3-fold, respectively). Expressions were increased further in middle-aged mice (1.103 ± 187-fold; 4.4 ± 0.7-fold) and further still in elderly mice (1.218 ± 168-fold; 6.9 ± 0.3-fold). IL-10 and heme oxygenase-1 gene expressions were also elevated postoperatively in young mice (4.8 ± 0.5-fold and 13.0 ± 1.3-fold, respectively) and were further increased in middle-aged mice (7.5 ± 0.6-fold; 21.8 ± 3.2-fold). However, inductions in elderly mice were significantly blunted (5.8 ± 0.9-fold; 16.9 ± 0.8-fold). There is both an age-dependent increase in the proinflammatory mediator expression and an age-dependent decrease in anti-inflammatory mediator expressions following minor insult to the bowel. Such imbalances between pro- and anti-inflammatory mechanisms may form the basis for increased susceptibility to ileus and for the increased severity and duration of ileus observed in the elderly.

Nevertheless, studies of aging animals and humans have reported the slow accumulation of generalized degenerative changes in the structural and metabolic integrity of many organ systems, including the gastrointestinal tract (7, 11, 12, 41). It has been proposed that membrane lipid peroxidation injury and mitochondrial and nuclear DNA mutations, accumulated from a lifetime of exposure to reactive oxygen species, leads to a decline in stress tolerance and in the capacity to initiate cellular and tissue repair (1, 12). Whether a direct consequence or a separate entity, elevated basal levels of activated transcription factors linked to proinflammatory cytokine production have also been reported with advancing age (25). These findings suggest that aging organ systems experience a progressive loss of functional reserve that coexists with an increase in proinflammatory status. While in health, normal function is maintained; however, a relatively minor insult can lead to inflammation and local tissue injury that rapidly overcomes this reserve, ending in functional impairment that seems out of proportion to the initiating event.

Observations in the clinic suggest that the gastrointestinal tract is impacted by this phenomenon, where, commencing as early as age 50, susceptibility to postoperative ileus increases with patient age. This is particularly evident in the elderly (aged 64 years and older), where even minor incursions into the abdominal cavity lead to significant ileus and where the clinical manifestations of ileus are more severe in magnitude and prolonged in duration. Such prolonged loss of normal motility patterns of the gastrointestinal tract favors bacterial overgrowth of the bowel with increased risk of impaired mucosal barrier function. The translocation of antigen, toxins, and bacteria from the intestinal lumen into the systemic circulation could potentially contribute to the increased incidence of systemic inflammatory response syndrome, sepsis, and multiple organ failure observed in this age group (33). The clinical management of the elderly presents an exceptional challenge to physicians because multiple comorbidities and their therapies can place these patients at accelerated risk for the most severe complications. A better understanding of inflammatory processes in the elderly will provide useful insights for clinical intervention in this patient population.

Using a model of postoperative ileus in young adult rodents, we have shown that mild compression of the small intestine, a technique designed to mimic "running" of the bowel routinely performed in the clinical setting, leads to the development of significantly impaired smooth muscle contractility and delayed
gastrointestinal transit. Manipulation of the bowel activates macrophages normally resident within the intestinal muscularis, leading to the induction of a molecular inflammatory response (21) characterized by the release of proinflammatory cytokines (IL-6, IL-1β) and chemokines (monocyte chemotactic protein-1) and by the expression of adhesion molecules (ICAM-1) on the intestinal microvasculature (16–18, 40, 43). Recruitment of a cellular infiltrate from the systemic circulation then ensues (19). Both resident and recruited leukocytes continue to release cytokines, chemokines, cyclooxygenase-2 (COX-2)-derived prostaglandins, and inducible nitric oxide synthase (iNOS)-derived nitric oxide (13, 20, 38). Many of these mediators have direct effects on the neuromuscular apparatus within the gastrointestinal tract, leading to disruption of coordinated contractile activity (4, 20, 32, 38). The enhanced expression of anti-inflammatory mediators, such as IL-10 and heme oxygenase (HO)-1, then ensues (29) to regulate the inflammatory response and initiate restoration of homeostasis.

A balanced interaction between pro- and anti-inflammatory mechanisms is essential, both to develop an appropriate inflammatory response to initiate protection, repair, and healing and to limit the magnitude and duration of inflammation to prevent injury to healthy tissues. In this study, we hypothesized that a shift in the balance between pro- and anti-inflammatory mechanisms underlies the increased susceptibility to postoperative ileus in the elderly. We employed a modified surgical protocol to determine whether an age-dependent increase in susceptibility to postoperative ileus could be reproduced in a murine model. Surgically induced delays in gastrointestinal transit were measured as an index of the severity of ileus. The expression of pro- and anti-inflammatory mediators during the development of postoperative ileus was evaluated to identify those that are altered with age.

MATERIALS AND METHODS

Animals. C57Bl/6 male mice were obtained from the National Institute on Aging breeding colony at Harlan Sprague Dawley (Indianapolis, IN). The protocol was approved by the University of Pittsburgh Institutional Animal Care and Use Committee. Mice were housed in a pathogen-free facility that is accredited by the American Association for Accreditation of Laboratory Animal Care and complies with the requirements of humane animal care as stipulated by the U.S. Department of Agriculture and the Department of Health and Human Services. Animals were maintained on a 12:12-h light-dark cycle and were provided with commercially available rodent chow and tap water ad libitum.

Experimental groups and operative procedures. The experimental protocol described in this section was designed specifically to determine whether, like humans, aging mice exhibit an increased susceptibility to the development of postoperative ileus in response to a mild disturbance to the bowel. We have demonstrated that the severity of postoperative ileus arising from abdominal surgery is closely correlated with the intensity of the disturbance to the bowel during surgery (22), and we have developed a standardized protocol for the induction of a clinically relevant degree of ileus. In this protocol, mice are anesthetized by isoflurane inhalation, a laparotomy is performed, the bowel is everted onto moistened sterile gauze, and the entire small bowel is then gently compressed along its entire length between moistened cotton applicators. Compression does not cause capillary breakage or stretching of mesentery and is designed to simulate “running” of the bowel that is often performed in the clinical setting. The bowel is repositioned in the abdominal cavity, and the incision is closed. Duration of the procedure is ~20 min. This “standard” manipulation results in significantly delayed transit with retention of the transit marker within the proximal one-third or less of the mouse gastrointestinal tract (29). In the current study, the intensity of bowel manipulation was modified to detect age-dependent differences in the susceptibility to postoperative ileus. In this case, the small bowel underwent “light” manipulation (LM), whereby cotton-tipped applicators were lightly rolled, without compression, over the surface of the bowel. This degree of bowel disturbance did not significantly delay transit in young adult mice and provided a background on which to detect alterations in transit with increasing age.

Experimental endpoints were determined in three age groups: 2–3 mo (young adult), 12–15 mo (middle-aged), and 24–28 mo (elderly). Study groups consisted of operated animals that underwent anesthesia, laparotomy, and LM. Age-matched, unoperated mice served as controls. We have shown (22) that sham surgery (anesthesia and laparotomy only) results in a low level of induction of proinflammatory mediator expression but that it does not result in functional impairment. A second group of elderly mice underwent sham surgery to determine whether this age group is more sensitive to developing functional impairment from anesthesia and laparotomy alone.

Functional studies. Intestinal transit was measured in controls, manipulated animals, and sham-operated animals 24 h postoperatively by evaluating the intestinal distribution of nonabsorbable fluorescein-labeled dextran (molecular weight = 70,000). Animals were fed labeled dextran orally (100 μl of 6.5 mg/ml stock solution). Sixty minutes after administration, the animal was killed and the entire bowel from stomach to distal colon was collected. The contents of the stomach, small bowel (divided into 10 segments of equal length), cecum, and colon (3 equal segments) were pelleted, and aliquots of the cleared supernatant were read in duplicate in a multwell fluorescence plate reader (Gemini XPS; Molecular Devices, Sunnyvale, CA; excitation wavelength 530 nm and emission 590 nm). Data were expressed as the percentage of total fluorescence signal in each segment and were plotted in a median histogram. Gastrointestinal transit was calculated as the weighted average distribution (geometric center; GC) of labeled dextran along the gastrointestinal tract by using the following formula: GC = Σ(%total fluorescent signal per segment × segment number)/100, where higher values of GC indicate a more distal distribution.

Pro- and anti-inflammatory gene expression. Small intestinal muscularis externa was harvested 3 h postoperatively and was snap frozen in liquid nitrogen. Total RNA extraction was performed by using the guanidium-thiocyanate phenol-chloroform extraction method. RNA pellets were resuspended in RNAsecure resuspension solution (Ambion, Austin, TX), followed by removal of potentially contaminating DNA by treatment with DNase I (DNA-Free kit; Ambion). Aliquots (2 μl) of extracted RNA from each sample were quantified by spectrophotometry (ratio of 260/280 nm wavelengths) and were diluted to generate RNA stock solutions containing 40 ng/μl total RNA.

IL-6, COX-2, iNOS, IL-10, and HO-1 mRNA expressions were quantified 3 h postoperatively in duplicate by SYBR Green two-step, real-time RT-PCR with GAPDH as the endogenous reference. Aliquots of stock RNA were subjected to first-strand complementary DNA (cDNA) synthesis by using random hexamers (PE Applied Biosystems, Foster City, CA) and Super Script II (Life Technologies, Rockville, MD). Primers were designed according to published sequences and GenBank accession numbers by using Primer Express software (PE Applied Biosystems). Primer sources and sequences are summarized in Table 1.

PCR reaction mixture was prepared by using SYBR Green PCR Core reagents (PE Applied Biosystems). Each sample was estimated in duplicate by using the conditions recommended by the manufacturer. The reaction was incubated at 50°C for 2 min to activate uracil N-glycosylase, then for 95°C for 10 min to activate AmpliTaq Gold DNA polymerase, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min on an ABI PRISM 7700 Sequence Detection system (PE Applied Biosystems). Real-time PCR data were plotted as the ΔΔCt
Table 1. Primer sequences

<table>
<thead>
<tr>
<th>Primer</th>
<th>Source</th>
<th>Sequence, 5’ to 3’</th>
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<tr>
<td>Forward</td>
<td></td>
<td>GCCAGTACCTGTGCTCATGA</td>
</tr>
<tr>
<td>Reverse</td>
<td></td>
<td>TCAATCCAGAAACCCGTAAGA</td>
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<td>IL-6</td>
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<td></td>
<td>CTGGACGCAAACGCCTCTGA</td>
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<tr>
<td>Reverse</td>
<td></td>
<td>ACGCTGTTGTACACACACGGC</td>
</tr>
<tr>
<td>COX-2</td>
<td>GenBank NM_11198</td>
<td>GTGACGCGGGAAACTAGCTTCA</td>
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<td>Forward</td>
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<tr>
<td>Reverse</td>
<td></td>
<td>CACATTGCGGAGAGCTTACA</td>
</tr>
<tr>
<td>IL-10</td>
<td>GenBank M_37897</td>
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</tr>
<tr>
<td>Forward</td>
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<tr>
<td>Reverse</td>
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</tr>
<tr>
<td>Forward</td>
<td></td>
<td>GTGACGGTGTACCACACGGC</td>
</tr>
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<tr>
<td>Forward</td>
<td></td>
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</tr>
<tr>
<td>Reverse</td>
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<td>CACCGTGAGGAGGCTTACA</td>
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COX-2, cyclooxygenase-2; iNOS, inducible nitric oxide synthase; HO-1, heme oxygenase-1.

fluorescence signal vs. the cycle number to determine the threshold cycle (C<sub>T</sub>). Quantification of mRNA expression was normalized to the GAPDH reference gene and was calculated relative to control by using the comparative C<sub>T</sub> method (36).

To exclude PCR amplification of contaminating genomic DNA, RT-negative controls (samples containing RNA that was not reverse transcribed) were included in each PCR reaction. Gel electrophoresis was performed for the primers to confirm the absence of nonspecific bands and that the amplicons were of the expected size. Efficiency of primer amplification was performed for the primers to confirm the absence of nonspecific products and that the amplicons were of the expected size. Efficiency of PCR amplification of target cDNA was determined to ensure colinarity of primer amplification. Serial threefold dilutions of target cDNA were performed in triplicate. Standard curves were generated by plotting C<sub>T</sub> values against the relative input copy number. Slopes of −3.22 ± 0.2 (r<sup>2</sup> = 0.99) with corresponding efficiencies of 100 ± 5% were considered acceptable. Melting curve analysis was performed for each PCR reaction to ensure amplification of a single product.

Myeloperoxidase histochemistry. Muscularis whole mounts were prepared from the midcolon collected 24 h after treatment. Intact segments of midcolon were immersed in Krebs-Ringer bicarbonate buffer and were removed by fine dissection. The remaining tissue was placed in serum-free DMEM and was incubated for 24 h in a 37°C incubator containing air equilibrated with 5% CO<sub>2</sub>. IL-6 and PGE<sub>2</sub> were quantified in aliquots of culture medium by Quantikine colorimetric ELISA (R&D Systems, Minneapolis, MN), according to the manufacturer’s instructions. Total nitrate was measured as an index of nitric oxide production in aliquots of culture medium by using the Griess reaction. All values were normalized to tissue wet weight.

The expression of IL-10 and HO-1 protein was compared by using standard Western blot analyses. Small intestinal muscularis was harvested at 6 or 8 h postoperatively (peak expression of IL-10 and HO-1 protein expression, respectively) and was snap frozen in liquid nitro-
Total protein was extracted on ice by homogenization in 50 mM Tris·HCl lysis buffer (pH 7.4) containing 0.5% SDS and protease inhibitors. Aliquots of supernatant were analyzed for protein content by using the bicinchoninic acid method (Pierce Biotechnology, Rockford, IL). Kaleidoscope prestained molecular weight markers (Bio-Rad, Hercules, CA), mouse recombinant IL-10 (50 ng, Chemicon International, Temecula, CA), and rat recombinant HO-1 (50 ng; Stressgen Biotechnologies, Vancouver, BC, Canada), and sample aliquots containing 20 μg total protein were loaded onto freshly poured 12% SDS-PAGE gels and were electrophoresed under denaturing conditions. Paired gels were treated with GelCode Blue stain reagent (Pierce) to visualize equal loading of lanes. Proteins were transferred to Hybond-P transfer membrane (Amersham Biosciences, Piscataway, NJ) by using the Bio-Rad Transblot system. Nonspecific binding was blocked by using 5% bovine serum albumin, and membranes were incubated overnight with primary antibodies directed against IL-10 (1:1,000; Chemicon International) and HO-1 (1:2,500, Stressgen Biotechnologies). Specific protein bands were determined by using the ECL Western blot detection kit and were recorded on ECL Hyperfilm (Amersham Biosciences). Intensities of the 32-kDa band (HO-1) and the 36-kDa band (IL-10, active dimer) were determined by densitometry and were reported as relative change in band intensity for operated mice compared with unoperated controls.

RESULTS

General observations. All animals used in these studies exhibited no overt signs of significant disease. Although some elderly mice (~15%) had minor hindlimb stiffness indicative of arthritic changes, all were active, moved freely, and ate and drank normally. Approximately 10% of middle-aged mice and 90% of elderly mice had hypertrophied seminal vesicles, and all had greater amounts of intra-abdominal fat compared with young-adult mice. Occasionally, evidence of liver or other organ pathology was found during tissue harvest, and data from these animals were excluded from the study.

Gastrointestinal transit. Figure 1 shows the effects of laparotomy and LM on gastrointestinal transit among animals of different ages. In unoperated control animals of all age groups,
labeled dextran was distributed primarily within the distal small bowel 60 min after ingesting the dextran (Fig. 1A). Transit was markedly delayed in elderly mice that had undergone LM, where the preponderance of labeled dextran was found in the stomach and proximal small bowel.

Figure 1B summarizes the calculated GC values for all experimental manipulations. Higher values of GC indicate a more distal distribution of fluorescent signal and correspond to a more rapid transit. No differences were observed in GC values in unoperated controls among the three age groups. Sham surgery (data for elderly mice are shown) had no effect on transit. GC values in young mice that had undergone LM were unchanged compared with age-matched controls; however, a significant age-dependent decrease in GC was observed in middle-aged and elderly mice.

**Inflammatory cell infiltrate.** Our standard surgical manipulation (SM) of the small bowel or colon typically induces a massive cellular inflammatory response within the muscularis, whereby the onset and magnitude of the infiltrate correlates with the onset and severity of ileus (14). Histological analysis of MPO activity was used to quantify activated leukocytes infiltrating the intestinal muscularis from control and LM animals. In control specimens, MPO-positive cells were scarce in all age groups (≤1.7 ± 0.2 cells/×200 field of view). LM did not induce an inflammatory cell infiltrate in any age group (young, 1.9 ± 0.1; middle-aged, 1.8 ± 0.2; elderly, 2.3 ± 0.4 cells/×200 field). For comparison, consider the magnitude of the infiltrate obtained in young mice (76 ± 6.3 cells/×200 field) and elderly mice (84 ± 7.0 cells/×200 field) induced by standard manipulation of the small bowel (n = 4 per group).

**Prolonged mediator expression.** We have demonstrated consistently that the expression of proinflammatory mediators is upregulated early within the intestinal muscularis during the development of postoperative ileus and that there is a positive correlation with the degree of insult, magnitude of the inflammatory response, and severity of motility impairment (14, 15). In the current study, results from real-time RT-PCR analyses of mRNA expressions and results from protein analyses for the proinflammatory mediator IL-6 are shown in Fig. 2. In Fig. 2A, LM was shown to induce an increase in IL-6 message within the muscularis in all age groups. Data are presented as fold increase relative to age-matched naïve control mice. A marked increase in IL-6 message was observed in middle-aged mice compared with young animals; however, induction in elderly mice did not appear to be different from that in young animals. Comparison among age groups of
baseline mRNA expression for various mediators is shown in Fig. 2B. Data were calculated as fold increase relative to naïve young mice. Resting levels in middle-aged animals were not different from those in the young; however, basal IL-6 message was significantly greater in elderly mice compared with either young or middle-aged animals. Basal release of IL-6 protein was also significantly greater in elderly mice compared with the other age groups. When naïve young mice were taken to represent “normal” baseline values and fold increases in message were recalculated relative to the young controls, IL-6 mRNA levels in elderly mice were now comparable with those obtained in middle-aged animals (Fig. 2C). This observation was supported by analysis of IL-6 protein (Fig. 2D), where protein release from the intestinal muscularis was similar in middle-aged and elderly animals and both were significantly greater than that in young mice. Sham surgery in elderly mice resulted in the induction of IL-6 mRNA and protein to a level that was comparable with that in young mice that had undergone LM.

Prostaglandins derived from COX-2 and nitric oxide derived from iNOS are potent inhibitors of intestinal smooth muscle contractility (Fig. 3). Figure 3A shows a significant age-dependent increase in the expression of COX-2 mRNA in response to LM, calculated relative to levels in young naïve animals. This was not accompanied by a corresponding increase in the production of PGE₂ (Fig. 3B). A significant increase in baseline PGE₂ production was observed in elderly mice and likely is a reflection of the slightly higher baseline COX-2 mRNA expression (Fig. 2B) seen in this age group. However, the response was modest compared with the three- to fourfold increase typically seen following a standard manipulation in young animals (29). Baseline iNOS expression was significantly greater in elderly mice (Fig. 2B). LM did not induce iNOS mRNA expression in any age group (Fig. 3C), with the apparent increase in induction in elderly mice being a direct reflection of increased baseline expression. The production of NO at baseline and in response to LM was unchanged among age groups (Fig. 3D). For comparison, NO production in young mice in response to standard manipulation approaches 100 μM/100 mg tissue (29). Sham surgery did not result in the induction of COX-2 or iNOS mRNA (data not shown).

**Transcription factor activation.** The induction of the proinflammatory events associated with postoperative ileus is associated with the activation of the JAK-STAT and NF-κB sig-

![Figure 4](http://ajpgi.physiology.org/)

**Fig. 4.** Effect of age on signal transducer and activator of transcription 3 (STAT3) and NF-κB activation in response to LM. EMSA was performed using 20 μg of protein extracted from muscularis externae from naïve control mice (C) and from mice having undergone LM. **A:** radiolabeled high-affinity serum-inducible element duplex oligonucleotide was used to characterize STAT3 protein activation. **Top** shows representative samples from naïve controls and LM-operated mice from the 3 age groups. Band intensity is clearly increased in response to LM. Densitometer analysis at **bottom** shows that baseline STAT3 band density was elevated in response to LM in all age groups. The response in elderly mice was significantly greater than young and middle-aged animals. LM in all age groups vs. response in naïve young mice, *P < 0.05 and **P < 0.01. Elderly control vs. young control, †P < 0.05. **B:** radiolabeled α-dCTP was used to characterize NF-κB protein activation. Top shows representative samples from same animals as in **A.** Baseline NF-κB expression was unchanged among age groups. LM induced a small increase in band density only in elderly mice. LM vs. control in elderly mice, *P < 0.05. Data are means ± SE; n = 6.
naling pathways (37, 43). The effect of LM on transcription factor activation for the three age groups is shown in Fig. 4. Activation of STAT3 homodimers was significantly elevated in the manipulated small bowel compared with control in all age groups, and an age-dependent increase in the magnitude of activation was observed. In addition, the baseline levels of activated STAT3 were significantly elevated in elderly mice. LM did not activate NF-κB in young or middle-aged mice but caused a small, though significant, activation in elderly mice. No differences in baseline activation of NF-κB were measured among age groups.

Anti-inflammatory mediator expression. The IL-10 mRNA expression profile is shown in Fig. 5. LM resulted in significant induction of message in young mice and a greater induction in middle-aged animals. Message induction in the elderly mice appeared to be blunted and was not significantly different from either that in young or middle-aged animals. A similar expression profile for HO-1 mRNA is shown in (Fig. 6). These levels of IL-10 and HO-1 message induction are well below previously published responses, where increases evoked by standard manipulation are typically in excess of 15- and 40-fold, respectively (29). Western blot analysis of IL-10 protein mirrors that of the message profile, where protein expression in the elderly mice was significantly less than that in middle-aged animals and was not different from that in young animals. However, HO-1 protein expression did not mirror the mRNA expression profile, instead showing a clear age-dependent decrease in the synthesis of HO-1 protein. No differences were found in basal IL-10 and HO-1 message or protein expression among the three age groups, nor was expression induced by sham surgery (data not shown).

DISCUSSION

In the current study, we observed that mice that underwent abdominal surgery exhibited increased susceptibility to the development of postoperative ileus with advancing age, with elderly mice exhibiting the greatest delays in gastrointestinal transit. Consistent with studies of healthy humans (27, 35), no age-related differences in mouse baseline gastrointestinal transit were observed. However, age-dependent differences in the expression profiles of pro- and anti-inflammatory mediators were observed, both in naïve animals and in those having undergone abdominal surgery.

Evidence for a heightened proinflammatory status in the elderly mouse intestine at rest was provided by comparisons among age groups of baseline gene expression in the mouse intestinal muscularis. Message levels of the proinflammatory mediators IL-6, COX-2, and iNOS were significantly elevated in the oldest age group, whereas no differences were observed for the anti-inflammatory mediators IL-10 and HO-1. Although this was not accompanied by an increase in iNOS activity, baseline COX-2 activity was elevated in elderly mice, resulting in a modest increase in PGE2 production. It was clear that basal IL-6 mRNA expression and protein release were considerably higher in the muscularis of elderly mice. Measurements of elevated plasma IL-6 protein levels in aging animals and humans are cited as support for the theory that advancing age is associated with an enhanced proinflammatory state (10).
Overflow of IL-6 into the systemic circulation from aging skeletal muscle and other tissues is thought to account for the increased plasma levels reported in this age group (9). Our data suggest that the intestinal muscularis may be an additional source of increased circulating IL-6. In addition, EMSA analysis shows increased basal STAT3 transcription factor binding. IL-6 exerts many of its downstream effects in smooth muscle tissues via activation of the JAK-STAT signaling pathway (44), and these observations are consistent with published reports of elevated basal levels of activated transcription factors associated with pro-inflammatory cytokine production in aging tissues (25). Although it was clear that none of these factors was sufficient to cause functional impairment in unoperated mice, the presence at rest of elevated IL-6 and PGE2 protein and of activated cytokine-associated transcription factors suggests that inflammatory mechanisms exist in a heightened state of readiness. Under these conditions, the aging gastrointestinal tract can be considered to exist in a primed pro-inflammatory state, quick to respond to a relatively minor insult with a robust, perhaps unnecessarily robust, inflammatory response.

Evidence for an exaggerated inflammatory response to a mild stimulus was found when LM of the small bowel was applied with the same intensity in all age groups. In middle-aged and elderly mice, the expression of IL-6 message and protein reached levels comparable with those achieved by LM in young adult mice that exhibit the functional and molecular responses typical of fully developed ileus (29). This was accompanied by an age-dependent increase in STAT3 binding, lending further support for an enhanced inflammatory response. In addition, age-related changes in anti-inflammatory mediator production were observed. The induction of IL-10 and HO-1 message occurred in all age groups, with the greatest increase observed in middle-aged mice. In elderly mice, however, message induction did not exceed that obtained in young animals. The expression pattern of the active IL-10 dimer showed good correlation with message, indicating that the capacity to mount IL-10-mediated anti-inflammatory mechanisms becomes blunted with age. Furthermore, the expression pattern of HO-1 protein did not correlate with that of message, where protein expression was highest in young mice and progressively decreased with age. IL-10 has a wide spectrum of biological effects on lymphoid and myeloid cells, where one of its known functions is to inhibit the production of pro-inflammatory cytokines, including IL-6 (31). In addition, it has become increasingly apparent that the activity of HO-1, the rate-limiting enzyme in heme catabolism, has important anti-inflammatory and cytoprotective effects under a variety of acute and chronic inflammatory conditions (reviewed in Ref. 5), including those within the gastrointestinal tract (42). Redox cycling between two of its major metabolic byproducts, biliverdin and bilirubin, confers potent anti-oxidant properties to the activity of HO-1 (6). An age-dependent decline in the induction of human heat shock proteins, including HO-1, is associated with increased oxidative injury (3). Moreover, certain anti-inflammatory effects of IL-10 have now been shown to be dependent on the activity of HO-1 (26). Thus a disruption in the balanced production of IL-10 and HO-1 could result in a reduced capacity to regulate inflammatory mediator production and to counter oxidative stress. Such aging-related changes in the metabolic properties of the gastrointestinal tract have been reported, where aging is associated with a progressive decline in the capacity to counter oxidative stress (1, 12). Together, our data suggest that the increasingly exaggerated pro-inflammatory response seen with advancing age may be attributed to a progressive shift in the balance between pro- and anti-inflammatory cytokines.
anti-inflammatory mechanisms correlated with the age-dependent increase in severity of the impairment of gastrointestinal transit in this model of postoperative ileus.

The mechanisms underlying the development of postoperative ileus are complex, involving central neural reflexes, hormonal influences, local molecular inflammatory responses, and the recruitment into the intestinal muscularis of activated immune cells (2, 22–24, 43). An unexpected finding in this study was that middle-aged and elderly mice developed significant ileus in the absence of immune cell recruitment. This is in contrast to previous studies (14) where the onset and severity of ileus was consistently found to correlate temporally with the onset and magnitude of the inflammatory cell infiltrate. The paradigm believed to underlie inflammatory mechanisms in postoperative ileus is that inflammation is driven by the activation of macrophages that are normally resident within the muscularis, giving rise to a molecular inflammatory response with the expression of cytokines, chemokines, and adhesion molecules (22, 43). This in turn leads to a cellular inflammatory response subsequent to the recruitment of circulating leukocytes (15). The enhanced release from these cells of prostaglandins derived from the activity of COX-2 and nitric oxide derived from the activity of iNOS has been linked to the disruption of coordinated gastrointestinal contractility (13, 19, 38). Interventions that reduce the magnitude of the cellular infiltrate markedly attenuate ileus by reducing COX-2 and iNOS gene expression and the production of prostaglandins and nitric oxide (15, 30, 40). In the current study, however, there was both an absence of an inflammatory cell infiltrate and a lack of significant prostaglandin and nitric oxide synthesis. In addition, there was little activation of NF-κB, a transcription factor linked to the induction of iNOS and COX-2 expression during gastrointestinal inflammatory events (28, 39). This was not merely a failure of immune cell trafficking in older animals, because both young and elderly mice exhibited similar numbers of infiltrating leukocytes following standard surgical manipulation. These data show that the light disturbance to the bowel used in this study was insufficient to trigger a cellular inflammatory response and demonstrate that mechanisms exist within the muscularis that are sufficient to cause dysmotility in the absence of cellular inflammation.

The identification of the specific mechanisms that produce ileus in the absence of inflammation is beyond the scope of this manuscript. However, hormonal influences or the stimulation of central inhibitory neural reflex pathways remains a possibility. In addition, the activation of the resident immune cell population alone may be sufficient to cause ileus. IL-6 is produced by resident macrophages residing within the intestinal muscularis (43), and IL-6 protein was highly abundant within the intestinal smooth muscle explants of middle-aged and elderly mice after LM. Proinflammatory cytokines can alter smooth muscle contractility, as demonstrated by studies in vitro where exposure of gastrointestinal smooth muscle to IL-6 or IL-1β decreased contractility in a dose-dependent fashion by inhibiting the release of acetylcholine from neurons of the enteric nervous system (4, 32). In addition, it is conceivable that the large quantity of IL-6 protein produced in aging mice can interfere with neuromuscular communication.

In conclusion, the results presented here suggest that, although gastrointestinal function is well preserved in the healthy elderly mice, conditions exist that cause the gut to be less tolerant of stressors. This is due in part to the presence of persistent low-grade inflammation, leaving the gut in a “primed” pro-inflammatory state, which is accompanied by an aging-related decline in the capacity to mount an appropriate anti-inflammatory response. Under these conditions, even a relatively minor insult results in the development of significant gastrointestinal ileus. Such imbalances between pro- and anti-inflammatory mechanisms may form the basis for increased susceptibility to ileus and for the increased severity and duration of ileus observed in elderly humans.

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REFERENCES


