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MECHANISMS OF SMOOTH MUSCLE RESPONSES TO INFLAMMATION

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Gut smooth muscle is arranged simply into two distinct layers, circular and longitudinal, with the myenteric plexus interspersed between these layers. Individual smooth muscle contractility requires communication between the two layers to generate motor patterns, which must be coordinated along the gut to result in effective propulsion and transit. Smooth muscle contractions provide a major driving force for movement of luminal content along the gastrointestinal (GI) tract. The influx of calcium (Ca^{2+}) through voltage-dependent L-type Ca^{2+} channels is key to the initiation of contraction. These channels are regulated by mechanical stimuli working through stretch-activated channels as well as agonists acting through G-protein coupled receptors (reviewed in¹). The increased levels of intracellular calcium (Ca^{2+}) ions, derived from extracellular, and augmented from intracellular sources, are bound to calmodulin and the calcium-calmodulin complex, which then binds to myosin light chain kinase (MLCK) and activates it. Activated MLCK phosphorylates MLC_{20} and promotes cross-bridge cycle between actin filaments and myosin head, which results in smooth muscle contraction. Myosin light chain phosphatase (MLCP) reverses the effect of MLCK resulting in smooth muscle relaxation (reviewed in^{1, 2}). Acetylcholine is a major neurotransmitter in the enteric nervous system and binds to both nicotinic and muscarinic receptors (MR). M3 receptors (M3R) are located on both smooth muscle and interstitial cells of Cajal³ and are coupled to G α_q and regulator of G-protein signaling-4 (RGS4), which controls the magnitude and duration of G α_q signaling. Recovery of smooth muscle is dependent in part on restoration of membrane potentials through opening of potassium (K^+) channels, a number of which are expressed on smooth muscle and are also targets of inhibitory agonists.

Patterns of contractions differ between the interdigestive period, which is characterized by the migrating motor complex (MMC), and the postprandial period, which features coordinated segmental and peristaltic contractions that facilitate digestion and absorption of nutrient, fluid and electrolytes. Dysmotility, therefore, may be the consequence of changes in the properties of smooth muscle itself, the degree or nature of the control exerted by

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nerves and hormones, the microenvironment, as well as the balance of cellular mediators present constitutively or in response to stimulation. The mechanisms responsible for these changes in these factors are variable, but inflammation arguably plays a major role in the abnormal motility associated with a number of GI pathologies. In this review we will discuss the various types of inflammation and the established as well as emerging mechanisms of inflammation-induced changes in smooth muscle morphology and function.

DEFINING THE INFLAMMATORY RESPONSE

The word “inflammation” is derived from the Latin *inflammare* meaning to ignite or set on fire. Aulus Cornelius Celsus, in the first century AD, initially described what are now considered the classical signs of acute inflammation: *rubor* (redness), *calor* (heat), *tumor* (swelling) and *dolor* (pain). In the early nineteenth century, the Polish physician/scientist Rudolph Ludwig Carl Virchow, contributed a fifth feature, *functio laesa* (restricted function) and intuited the “degenerative character of inflammation” relegating the classical signs to symptoms rather than causes. Of greater importance, however, was Virchow’s recognition of the importance of the *irratio* or initiating factor, proposing that inflammation was a process rather than a single entity. He was also the first to make the connection between the inflammatory microenvironment and cancer. The identification of hematopoietic cells involvement reinforced the cellular basis of inflammation, and the mechanisms involved in the inflammatory process remain an active area of investigation

Definitions of inflammation vary depending on the initiating factors and may lead to confusion as illustrated by the comparison of inflammation induced by chemical agents to that induced by pathogens including *Citrobacter rodentium* or *Trichinella spiralis*. A general concept is that the specific characteristics and effects of inflammation can be linked to the nature of the infiltrate and the associated mediators, which are dictated predominantly by the immune environment. Enteric infections are associated with the more classic polarization towards type 1 (Th1 cytokines) response that is needed for clearance of bacteria and viruses. The IL-17/Th17 cells expands this response but also have an emerging role in mucosal homeostasis⁴. In contrast, the type 2 (Th2 cytokines) immune responses are required for expulsion of extracellular pathogens like helminths and for allergic and asthmatic reactions in the lung. The Th2 cytokines are often termed “anti-inflammatory” but this description is misleading as it refers primarily to their ability to down-regulate cytokines in the Th1/Th17 pathway rather than an absence of inflammatory effects, particularly in the lung. GI inflammatory pathologies often feature a mixed profile of cytokines or a shift in profiles as the disease progresses from acute to chronic. Thus, inflammation is best viewed as a spectrum of changes that begins as a protective innate immune response to specific triggers and that progresses to chronic inflammation associated with a shift in the nature of infiltrating cells, the amplitude of the stimuli, and/or the size of the affected area.

IMMUNE CELLS, INFLAMMATORY MEDIATORS AND SMOOTH MUSCLE

The initiating event plays a major role in directing the inflammatory response. Although the first site of inflammation in the intestine is typically the mucosa, where microbes are generally encountered, events in the mucosa elicit changes in smooth muscle function

through activation of resident immune cells and recruitment of new cells that alter the local environment. The antigen presenting cells (APC), macrophages and dendritic cells, are important for the transition from innate to adaptive immune responses. Cytokines may regulate smooth muscle function by directly acting on smooth muscle cells, as well as indirectly through released mediators from other immune or non-hematopoietic cells. The previous dominant role assigned to T lymphocytes in shaping the immune response is being challenged by the emerging contribution of innate immune cells, such as macrophages, to the inflammation-induced changes in smooth muscle function. These infiltrating cells play a vital role in the changes in smooth muscle morphology (e.g. hypertrophy) that impact contractility⁵. In addition, smooth muscle is not a passive bystander during inflammation and our knowledge of molecular signaling pathways that control smooth muscle function, as well the contribution of the immune mechanisms to smooth muscle homeostasis, has expanded greatly in recent years. Studies in airway smooth muscle suggest that the responses to inflammation may be common to smooth muscle in general and encourage application of this information to GI smooth muscle⁶. Smooth muscle cells express receptors for a number of cytokines and chemokines, and activation of these receptors has both acute and long-lasting effects on function. Inflammation can alter ion channel activity and expression or activation of intracellular messengers or transcription factors that regulate genes that impact smooth muscle function. There are excellent reviews on the effect of inflammation on enteric nerves showing a general increase in sensitivity to stimulation as well as changes in the number and/or phenotype of enteric neurons^{7, 8}, and this area is beyond the scope of this review.

Type 1 immunity and smooth muscle

There is a wealth of information on induction of the type 1 response, which is characterized by upregulation of IL-12, IFN- γ , TNF α , and IL-1 and influx of hematopoietic cells, primarily neutrophils and macrophages. Bacterial products such as lipopolysaccharide (LPS) activate toll-like receptors (TLR) on epithelial cells, APC and other immune cells. TLR are linked to intracellular signaling adapter molecules such as MyD88 and activation of the canonical target, NF κ B, an essential regulator of inflammatory gene expression. STAT4 and T-bet are required for the full development of the type 1 response by transducing IL-12 and IL-23 signals into Th1 and Th17 cell differentiation and IFN- γ production. The effects of inflammation on smooth muscle induced by postoperative ileus, intestinal manipulation, chemical induction of inflammation using DSS or TNBS, or mesenteric ischemia reperfusion generally result in smooth muscle hypocontractility⁹⁻¹¹. This may be associated with decreased intestinal transit, which in the small intestine can lead to bacterial overgrowth. Reduced contractility of smooth muscle is also reported in patients with inflammatory bowel disease, which includes both ulcerative colitis and Crohn's disease^{12, 13}.

Smooth muscle responds directly to IL-1 β , TNF α , and IFN- γ via surface receptors^{14, 15} or, in the case of nitric oxide, via activation of intracellular molecules¹⁶. Smooth muscle cells treated with IFN- γ express MHC and can activate T cells¹⁷ as well as increased T cell proliferation¹⁸. The classic cytokine, IL-1 β , was among the first to be implicated in inflammatory bowel diseases and its role has expanded since to other cell activities

including proliferation and promotion of Th17 differentiation. IL-1 β increases smooth muscle proliferation, an effect that is amplified in the presence of IFN- γ and T cells¹⁸. Recent insights into the control of IL-1 β release show the important contribution of “inflammasomes”, which regulate the activity of caspase-1 to drive proteolytic processing of cytokines including IL-1 β cleavage from pro-IL-1 β ¹⁹. Inflammasomes are activated by a number of pathogens through pattern recognition receptors, as well as signals derived from damaged or apoptotic cells. This illustrates the importance of the integrated response to inflammation that leads to increased levels of IFN- γ and IL-1 β that have direct effects on smooth muscle.

The contribution of smooth muscle to the pathogenesis of GI inflammatory pathologies has been overshadowed by the tremendous focus on therapies that target the immune system. This should be reconsidered perhaps, in light of results showing that smooth muscle can be induced to generate a number of inflammatory mediators including chemokines that can modulate the influx of leukocytes. TNF α binds to both TNFR1 and R2 on smooth muscle, leading to activation of NF κ B and expression of a number of chemotactic genes, especially MCP-1, IL-8 and ICAM-1²⁰. Smooth muscle lacks receptors for several cytokines such as IL-12 and its transcription factor, STAT4. TLR2, TLR4 and NF κ B are all expressed on smooth muscle, which can be induced to generate inflammatory mediators as well as reactive oxygen species that are proposed to contribute to reduced smooth muscle contractility^{21, 22}. Mechanical distortion of smooth muscle in the form of static strain both increased the generation of NOS-2, COX-2 and IL-1 β and amplified the LPS-induced release of NOS-2 and IL-1 β ²³. Cytokines generated by Th17 cells are prominent in microbial infections, ischemia/reperfusion injury experimental models of colitis as well as in clinical IBD. There are receptors for IL-17, IL17-RA and IL-17RB on smooth muscle that are linked to enhanced IL-1 β -mediated CXCL-8 release from airway smooth muscle cells, yet the role of IL-17 in smooth muscle function in the GI tract is relatively unexplored; however, incubation of isolated smooth muscle cells with IL-17 enhanced contractility to acetylcholine²⁴.

There are few resident inflammatory cells in the muscle layers with the exception of macrophages, which are distributed in dense networks surrounding the myenteric plexus and the smooth muscle cells⁹. During type 1 responses, resident macrophages and influxed neutrophils are a source of MCP-1 that recruits additional macrophages to become classically activated (M1). M1 macrophages are characterized by inducible nitric oxide synthase (NOS-2). During inflammation, these resident and recruited cells also become a source of a number of mediators that alter smooth muscle function including IL-1 β , nitric oxide (NO), IL-6, and TNF- α . The M1 macrophage generation of large amounts of NO has potent inhibitory effects on smooth muscle. Both NO and MCP-1 also up-regulate the expression of ICAM-1 that promotes neutrophil infiltration^{25, 26}. M1 generation of NO and neutrophil influx into the muscle layers of intestinal ischemia/reperfusion, hemorrhagic shock, and ileus are important factors in the accompanying reduced smooth muscle contractility²⁷ as limiting the influx or activity of these cells diminishes the effect of the inflammatory stimuli on smooth muscle contractility. M1 macrophage production of insulin-like growth factor-1 (IGF-1) and transforming growth factor- β (TGF- β 1) play a role in the hypertrophy or hyperplasia of smooth muscle²⁸.

Type 2 immunity and smooth muscle

The type 2 immune response features increased expression of IL-4, IL-5, IL-9, and IL-13 and influx of basophils, mast cells, eosinophils, and macrophages^{29, 30}. The initiating events for the type 2 response are poorly characterized, but epithelial cells in the respiratory and GI tract are capable of producing IL-25 and IL-33, cytokines that have potent type 2-promoting activities^{31, 32}. In mice with enteric nematode infection, there is a characteristic hypercontractility of smooth muscle in response to various neurotransmitters or to nerve stimulation³³⁻³⁵. This stereotypic enhanced contractility is observed in a number of infections that preferentially infect the intestine and contributes to worm expulsion³⁴. Studies have found that IL-4 and IL-13 provide the driving force for the alterations in intestinal smooth muscle function during nematode infection, however, IL-13 is considered to be the major effector cytokine in the context of type 2 responses. Mice deficient in either IL-4 or IL-13 lack the hypercontractile response to infection while exogenous administration of IL-4 or IL-13 in mice mimics the intestinal smooth muscle hypercontractility observed in mice with nematode infection³⁴. There is growing appreciation of the role of epithelial cells in driving inflammatory responses that impact smooth muscle function. Recent studies further show that type 2-promoting cytokines IL-25, a member of the IL-17 family, and IL-33 are involved in modulation of smooth muscle contraction. Mice with IL-25 deficiency have attenuated smooth muscle response to nematode infection and impaired worm expulsion, whereas exogenous IL-25 induces a typical intestinal smooth muscle hypercontractility and promotes host immunity against nematode infection³¹. Similarly, exogenous administration of IL-33 also results in smooth muscle hypercontractility (Yang ZH et al, unpublished data).

There are a number of mechanisms by which IL-4 and IL-13 mediate their effects on smooth muscle. A major mechanism is through binding to receptors and activation of STAT6. IL-4 binds to type I IL-4 receptor that consists of IL-4R α and γ common chain, while IL-13 binds to type II IL-4 receptor that is composed of IL-4R α and IL-13R α 1 or the decoy receptor IL-13R α 2. Both types of IL-4R lead to the activation of STAT6 signaling pathway that turns on gene expression of various downstream molecules critical for the host immunity³⁰. Recent studies using transgenic mice that express or have deficiency in IL-4R α only on smooth muscle cells provide *in vivo* evidence for the importance of IL-4 and IL-13 effects on smooth muscle. Mice with IL-4R α deficiency only in smooth muscle cells have delayed worm expulsion after *N. brasiliensis* infection, low MR2 receptor expression, and attenuated smooth muscle response^{36, 37}. Mice engineered to overexpress IL-4R α only on smooth muscle showed smooth muscle hypercontractility in the lung airways in response to allergens or to IL-4/IL-13³⁸. Mice with IL-4R α deficiency only in smooth muscle cells fail to increase Th2 cytokines in response to helminth infection and have attenuated smooth muscle response^{36, 37} suggesting that direct cytokine activation of smooth muscle may play a role in induction of type 2 immunity.

Nematode infection results in a STAT6-dependent up-regulation of an array of receptors on smooth muscle such as M3, PAR-1, PAR-2, 5-HT_{2a}, which mediate the infection-induced hypercontractility to their respective agonists. The STAT6 pathway also plays an important role in IL-25- or IL-33-induced alterations in intestinal function. IL-25 binds to IL-25R, a

heterodimer consisting of IL-17RB and IL-17RA, leading to increased production of various type 2 cytokines such as IL-4, IL-5, and IL-13. Although IL-25 does not directly engage with STAT6, the downstream production of IL-13 acts through STAT6 pathway, such that the effects of IL-25 on smooth muscle function are abolished in IL-13^{-/-} and STAT6^{-/-} mice. IL-33 binds to a heterodimer receptor composed of ST2 and IL-1R accessory protein, leading to activation of NF- κ B and MAPKs pathways. Exogenous IL-33 induced an increased expression of IL-4, IL-5, and IL-13³⁹ but it remains to be determined whether the functional role of IL-33 on smooth muscle requires STAT6.

During enteric nematode infection a number of immune cells are recruited to the site of infection. Mast cells are a generic feature of type 2 responses in both the gut and lung and infiltrate both the mucosal and muscle layers. The mastocytosis is dependent on IL-3, IL-4 and IL-9, but not IL-13⁴⁰. Mast cells generate a number of cytokines including IL-4 and IL-13⁴¹. In response to the stimulation of type 2 cytokines, mast cells release cytokines, proteases (serine proteases and matrix metalloproteinases), and growth factors⁴² that participate in smooth muscle contraction or morphology. The location of mucosal mast cells near sensory afferents plays a role in neural hypersensitivity through release of serine proteases that can activate PAR-2 and generate leukotriene (LT) D₄^{43, 44}. Pretreatment with IL-13 increased the intracellular Ca²⁺ oscillations in airway smooth muscle, which are associated with enhanced contractility. In addition, Ca²⁺ oscillations in response to the mast cell mediator, LTD₄, were amplified in IL-13-treated airway smooth muscle through upregulation of the LTD₄ receptor. Similarly, LTD₄ enhanced the contractility of jejunal smooth muscle taken from mice treated with exogenous IL-4, an effect that was inhibited by an inhibitor of LTD₄ and abolished in mice deficient in 5-lipoxygenase⁴⁴, the enzyme responsible for LTD₄ production.

The ability of immune cells including macrophages and even T cells, to change their phenotype and activity in response to the local environment⁴⁵ will impact other cells in the area. Both resident and recruited macrophages accumulate in the smooth muscle and become alternatively activated macrophages (M2) by an IL-4/IL-13 and STAT6-dependent mechanism. These M2 macrophages play a key role for host protective immunity against nematode infection and are crucial for intestinal smooth muscle hypercontractility^{5, 29}. Like M1 macrophages, M2 macrophages also elaborate IGF-1, TGF- β 1, as well as arginase I that contribute to the characteristic hypertrophy or hyperplasia of smooth muscle induced by infection⁵.

INFLAMMATION-MEDIATED EFFECTS ON ION CHANNELS AND INTRACELLULAR SIGNALING MOLECULES

Given the importance of channels to smooth muscle function, there is also a growing interest in contribution of channelopathies to GI inflammatory diseases. Changes in smooth muscle contractility in experimental models of colitis have been linked to reduced activity and, in some species⁴⁶, decreases in the number of L-type Ca²⁺ channels⁴⁷⁻⁴⁹. In addition, there is evidence of post-translational modification of factors like c-src kinase that regulate Ca²⁺ channels⁵⁰, or increased activity or expression of K channels. A number of studies have focused on inflammation-induced changes in intracellular signaling. The decreased response

to muscarinic agonists such as carbachol in several models of colitis were attributed to a shift in the balance of myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP) activity resulting in a decrease in PKC-potentiated phosphatase inhibitor protein-17 (CPI-17)⁵¹. The activity of MLCP is primarily controlled by the phosphorylation state of myosin phosphatase target subunit 1 (MYPT1), one of the three subunits of MLCP. Rho kinase (RhoK) was the first protein that was discovered to phosphorylate MYPT1 at sites T696 and T853, and deactivate MLCP. In addition to interacting directly with MLCP, Rho kinase can exert its inhibitory effect by phosphorylating CPI-17 at T38. This, in turn, phosphorylates MYPT1 and results in Ca^{2+} sensitivity, which is defined as an increase in the force of the contraction at a given Ca^{2+} concentration. CPI-17 also contributes to Ca^{2+} sensitivity by binding the catalytic subunit of myosin phosphatase and inhibiting its activity, thereby enhancing MLC phosphorylation. There are decreased levels of CPI-17 in rodent models of colitis^{52, 53} and in patients with ulcerative colitis⁵⁴ that may contribute to the inflammation-induced hypocontractility⁵¹. In models of TNBS-induced inflammation, the increased expression of cytokines, such as IL-1, impair smooth muscle function by suppressing expression of the 1C1b subunit of Cav1.2b (L-type) calcium channels, CPI-17 and Gq⁵².

In vitro studies show that addition of IL-13 to the cultured airway smooth muscle cells increases contractility⁵⁵. In contrast to the reduced contractility in response to Th1 cytokines, IL-13 modulates intracellular calcium level in airway smooth muscle cells partly through CD38/cyclic adenosine diphosphate ribose pathway. IL-13 treatment of airway smooth muscle cells increased CD38 expression, and the calcium response to various agonists was significantly elevated. In addition to calcium channels, potassium channels play a critical role in contraction. Study on big potassium (BK) channels in bronchial smooth muscle cells indicated that IL-4, but not IL-13, activates BK channels in the presence of extracellular calcium. Conversely, others^{56, 57} indicate that in human bronchial smooth muscle cells, both IL-4 and IL-13 induce a STAT6-dependent up-regulation of the mRNA expression of RhoA, a small GTPase that is upstream of RhoK. Most of the effects of IL-13 on smooth muscle are mediated by STAT6 and the dependence of these changes on this transcription factor has not been investigated. Finally, cytokines may modulate activation G-protein coupled receptors (GPCR) on smooth muscle, thereby bypassing the need for the intermediate release of neurotransmitters. This is consistent with the upregulation of a number of GPCR in response to nematode infection^{14, 43, 58}.

INFLAMMATION-INDUCED CHANGES IN SMOOTH MUSCLE STRUCTURE

It is interesting that despite the opposing effects of Th1 and Th2 cytokines on smooth muscle contractility, both of these cytokine profiles result in smooth muscle hypertrophy and hyperplasia. Cytokines binding to their receptors influence intracellular signaling as well as expression of GPCR, and this can impact calcium handling and calcium sensitization. Perhaps the most convincing evidence is the smooth muscle remodeling that occurred in bronchial airways during asthma and in the GI tract in response to chronic inflammation. There is evidence for both type 1- and type 2-mediated remodeling of smooth muscle. Indeed, the development of fibrosis is thought to be a generic response to a variety of different inflammatory stimuli⁵⁹.

Abnormal smooth muscle function coupled with structural remodeling is considered a major factor in the pathology of Crohn's disease⁶⁰. The mechanisms involved in smooth muscle remodeling include macrophage release of growth factors such as TGF- β 1 and IGF. In Th1/Th17 dominated inflammation, smooth muscle production of IGF-1 stimulates proliferation and inhibits apoptosis resulting in hyperplasia, hypertrophy, and fibrosis^{28, 61}. A more recent study suggests a synergistic effect between TGF- β 1 and IL-13 in the formation of fistulae in Crohn's disease associated with an increase in the expression of IL-13R α 1⁶². We showed previously that there is a constitutive expression of IL-4R α , IL-13R α 1 and IL-13R α 2 in the smooth muscle cells of small intestine and colon indicating a potential direct role of IL-4/IL-13 on smooth muscle cells⁶³. Paradoxically, in the smooth muscle cells of intestine *N. brasiliensis* infection causes a down-regulation of IL-13R α 1, a functional receptor subunit for IL-13 with low affinity, but an up-regulation of IL-13R α 2, a decoy receptor for IL-13. As elevated levels of IL-13 simultaneously up-regulate the expression of IL-13R α 2^{63, 64}, these changes in IL-13R α 1 and IL-13R α 2 expression may serve to limit the effects as well as biological availability of IL-13 to avoid exaggerated IL-13 mediated responses⁶⁴. IL-13 is thought to play a role in the pathogenesis of ulcerative colitis and there is data indicating that binding of IL-13 to the decoy receptor, IL-13R α 2, is key to fibrosis in a murine model of chronic colitis. The mechanism is thought to be mediated by activation of AP-1 leading to secretion of TGF- β 1 and IGF-1⁶⁵. Thus, inflammation can induce both transient and persistent changes in smooth muscle proliferation and differentiation, illustrating the plasticity of smooth muscle as well as the potential for changes in smooth muscle morphology and phenotype leading to the formation of strictures and fibrosis.

NOVEL PLAYERS IN INFLAMMATION EFFECTS ON SMOOTH MUSCLE

In addition to the classic inflammatory pathways discussed earlier (Th1, Th17, Th2), and the involvement of cytokines receptors, other mediators can induce changes in smooth muscle function and be relevant for inflammatory diseases. In functional bowel disorders (e.g. IBS) or even during remission in IBD, signs of disease, such as diarrhea and abdominal pain, can be present even without classic hallmarks of active inflammation. Recent studies also demonstrated the relevance of extracellular proteases, of endogenous or exogenous origin, redox imbalance, or epigenetic mechanisms, to GI dysmotility and inflammation in the context of functional and organic disorders

Proteolytic pathways and proteases

Proteases and proteolytic pathways are important for extracellular matrix (ECM) degradation and inflammatory cell recruitment. During enteric infections, nematode-generated proteases are proposed to play a role in both the development and maintenance of the type 2 immune response⁶⁶. *N. brasiliensis* infection results in an immune-mediated up-regulation of PAR₁ and PAR₂ and contribute to the characteristic smooth muscle hypercontractility^{33, 43}. Proteases secreted by *Entamoeba histolytica* (cysteine proteases), damage enteric neurons, affecting, among others, smooth muscle function during infection⁶⁷. Proteases also contribute to type 1 responses. Recruitment of leukocytes to the intestinal smooth muscle contributes to the reduced contractility associated with upregulation of Th1 cytokines. The gelatinase family of metalloproteinases, particularly

MMP-9 and MMP-2, play an important role in the turnover and degradation of extracellular matrix proteins during cellular recruitment in inflammation. Mice treated with inhibitors of MMP-9, or MMP-9 deficient mice, have reduced recruitment of immune cells to the intestinal muscularis, preventing changes in smooth muscle function associated with post-operative ileus⁶⁸. There are a number of other proteases secreted by immune cells that are involved in intestinal inflammation related to smooth muscle dysfunction. Fibromuscular accumulation in the submucosa, which is recognized as part of the wound-healing response, is a histological hallmark of strictures in Crohn's disease. Mast cell derived chymase and cysteine proteases are actively involved in stricture formation in Crohn's disease⁶⁹. In addition to the classical effects of protease activity on ECM substrates, other proteases act on "non-conventional" substrates. ADAM15 (a disintegrin and metalloprotease) is characterized by both adhesive functions through its interaction with members of the integrin family, and protease properties. In the normal colon, ADAM15 is expressed, among other cell types, by pericryptic myofibroblasts co-expressing alpha-smooth muscle actin (SMA) and collagen IV. ADAM15 is expressed also by vascular myocytes in all layers of the intestinal wall as well as by nonvascular myocytes of the muscularis mucosae and muscularis propria. In IBD, ADAM15 is up-regulated strongly at the mRNA level and expressed only as an active form, delineating the relationship between mucosal regeneration and ADAM15 expression in pericryptic miofibroblasts⁷⁰.

Redox Imbalance, oxidative stress, and DNA integrity

Numerous studies have shown that persistent or recurring inflammation in the gut—*Helicobacter pylori* infection, ulcerative colitis, and reflux esophagitis—leads to cancer in the rapidly dividing epithelial cells. Little is known, however, about the effects of inflammation on DNA integrity in terminally differentiated cells, such as smooth muscle cells in the gut. Recent reports indicate that persistent inflammation impaired DNA integrity in the promoter region of *Cacna1c*, which encodes the pore-forming α_{1c} -subunit of $\text{Ca}_v 1.2b$ (L-type) calcium channels in smooth muscle cells⁷¹. This may be a mechanism for smooth muscle dysfunction in the absence of active inflammation. Oxidative stress in DSS inflammation impairs smooth muscle function by suppressing expression of the Gq protein. TNBS inflammation suppresses Gq because it also generates oxidative stress to the same extent as DSS inflammation⁷².

Epigenetic regulation

Cell and tissue homeostasis has been traditionally considered to be maintained and regulated by "linear" mechanisms, in which expression of genes was directly linked to the acquired phenotype, and only post-transcriptional and post-translational modifications were the factors able to introduce some degree of variability. In the past decade, novel mechanisms of regulation have been described, opening the epigenetic era, and being considered more and more important in organism development and pathogenic mechanisms involving inflammation⁷³. Recently, small non-coding RNAs, or miRNAs (miRNAs) were identified as key regulators in a wide variety of biological processes⁷⁴ and are now implicated in inflammatory diseases⁷⁵. In the past years a role for miRNAs in IBD was suggested, but the focus was on mucosal compartments, particularly barrier function dysregulation in inflammatory animal models^{76, 77}. Most of the studies on miRNAs and smooth muscle

function have been carried out on vascular or airways smooth muscle; however, many of the processes regulated by these miRNAs are generic features of general smooth muscle function, but this information was applied only recently to the regulation of intestinal smooth muscle homeostasis. These studies used mice with intestinal specific deficiency in Dicer, the ubiquitous endonuclease responsible for processing all cellular miRNAs, which results in loss of mature active miRNAs^{78, 79}. Dicer mice showed severe dilation of the intestinal tract associated with thinning and disruption of the smooth muscular layers. In addition, the authors showed that intestinal motility was dramatically decreased in association with extensive downregulation of smooth muscle contractile genes and transcriptional regulators^{80, 81}. Although more work needs to be carried out on the role of these novel regulatory mechanisms on intestinal smooth muscle function, they represent a potentially important target for novel molecular therapies for dysmotilities and other pathologies characterized by intestinal smooth muscle dysfunction.

CONCLUSIONS

Inflammation has a significant impact on smooth muscle. The nature of the initiating stimuli, the immune microenvironment as well as the profile of immune cells and their mediators play key roles in determining if the contractility is ultimately enhanced or diminished. A number of potential mechanisms are implicated including inflammatory mediator binding to receptors on smooth muscle leading to changes in the activity or number of ion channels or in the intracellular signaling molecules or activation of transcription factors that alter the expression of genes involved in smooth muscle function. Proteases contribute to smooth muscle response to inflammation by activation of receptors (e.g. PARs), recruitment of cells to the affected area, and remodeling of extracellular matrix. In addition, smooth muscle cells are not passive bystanders in inflammation and have the capacity to proliferate, differentiate, and elaborate chemokines that recruit cells and induce secretion of growth factor. Finally, there is a growing interest in the post translation regulation of smooth muscle by miRNAs to inflammation-induced changes in smooth muscle function and morphology.

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