Immunomodulatory Properties of Substance P

The Gastrointestinal System as a Model

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ABSTRACT: **Communication between nerves and immune and inflammatory cells of the small and large intestine plays a major role in the modulation of several intestinal functions, including intestinal motility, ion transport, and mucosal permeability. Neuroimmune interactions at intestinal sites have been associated with the pathophysiology of infectious and enterotoxin-mediated diarrhea and intestinal inflammation, including inflammatory bowel disease (IBD). During the past 20 years the neuropeptide substance P (SP) has been identified as an important mediator in the development and progress of intestinal inflammation by binding to its high-affinity neurokinin-1 receptor (NK-1R). This peptide, released from enteric nerves, sensory neurons, and inflammatory cells of the lamina propria during intestinal inflammation, participates in gut inflammation by interacting, directly or indirectly, with NK-1R expressed on nerves, epithelial cells, and immune and inflammatory cells, such as mast cells, macrophages, and T cells. SP-dependent activation of these cells leads to the release of cytokines and chemokines as well as other neuropeptides that modulate diarrhea, inflammation, and motility associated with the pathophysiology of several intestinal disease states. The recent development of specific nonpeptide NK-1R antagonists and NK-1R-deficient mice helped us understand the functional importance of the SP-NK-1R system in mediating intestinal neuroimmune interactions and to identify the particular cells and signaling pathways involved in this response. This review summarizes our understanding on the immunomodulatory properties of SP and its receptor in the intestinal tract with particular focus on their involvement in intestinal physiology as well as in the pathophysiology of several intestinal disease states at the *in vivo* and cell signaling level.**

KEYWORDS: **inflammation; colon; epithelial; neuropeptide**

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SUBSTANCE P IN THE INTESTINAL TRACT

Substance P (SP), an 11-amino acid neuropeptide, was originally isolated and purified by Chang and Leeman from bovine pituitary glands on the basis of its sialogogic activity.[1] SP is a member of the tachykinin family of peptides because it induces rapid smooth muscle contraction in guinea pig ileum and rat duodenum.[1] Other members of the tachykinin family, sharing common carboxyl terminal Phe-X-Gly-Leu-met-NH2 sequences in mammals, include neurokinin A and neurokinin B.[2] In mammals, tachykinins are produced by two genes, preprotachykinin-A (PPT-A) and preprotachykinin-B (PPT-B), and SP is a product of the PPT-A gene.[3,4] SP is localized in the central nervous system as well as in several peripheral tissues, including the entire length of the gastrointestinal tract as well as the colon. The main sources of SP in the gut include the myenteric and submucosal plexus, intrinsic sensory neurons, as well as sensory neurons originating from the dorsal root ganglia.[5,6] A newly identified gene, preprotachykinin C gene, encodes for the sequence of a new preprotachykinin protein designated hemokinin (HK) and produced primarily by hematopoietic cells.[7] HK binds with high selectivity to NK-1R and has similar *in vivo* potency to SP.[8] Like SP, HK is an 11-amino acid peptide having ~55% amino acid similarity to SP.[7,9]

SP RECEPTORS AND GUT DISTRIBUTION

The effects of SP are mediated by three different G-protein-coupled receptors (GPCRs), namely neurokinin (NK)-1, 2, and 3. SP binds with high affinity to NK-1 receptor (NK-1R), and with low affinity to NK-2 and 3 receptors. NK-1 receptors are present in both small intestine and colon of animals and humans and are localized in a variety of cells, including nerves, smooth muscle, immune cells, glands, endothelial cells, as well as epithelial cells.[10–15,16] Although NK-1 receptors have been associated with several intestinal pathophysiologic conditions (see below), NK-2 receptors have been linked mostly with circular muscle contraction,[17] and are localized in circular muscle and muscularis mucosae.[18] Although NK-2 receptors are present predominantly on smooth muscle and, like NK-1, can affect gut motility,[19] NK-3 receptors are expressed predominantly in neurons and can stimulate or diminish muscle contraction indirectly following SP binding to neuronal cells in the submucosal and myenteric nerve plexuses of the gastrointestinal tract.[20,21] NK-3 receptors also provide slow excitatory synaptic input to neurons in ganglia of the sphincter of Oddi.[22] Thus, both NK-2 and NK-3 receptors affect motility responses in the GI, but there is very little evidence that they are involved in neuroimmune interactions.

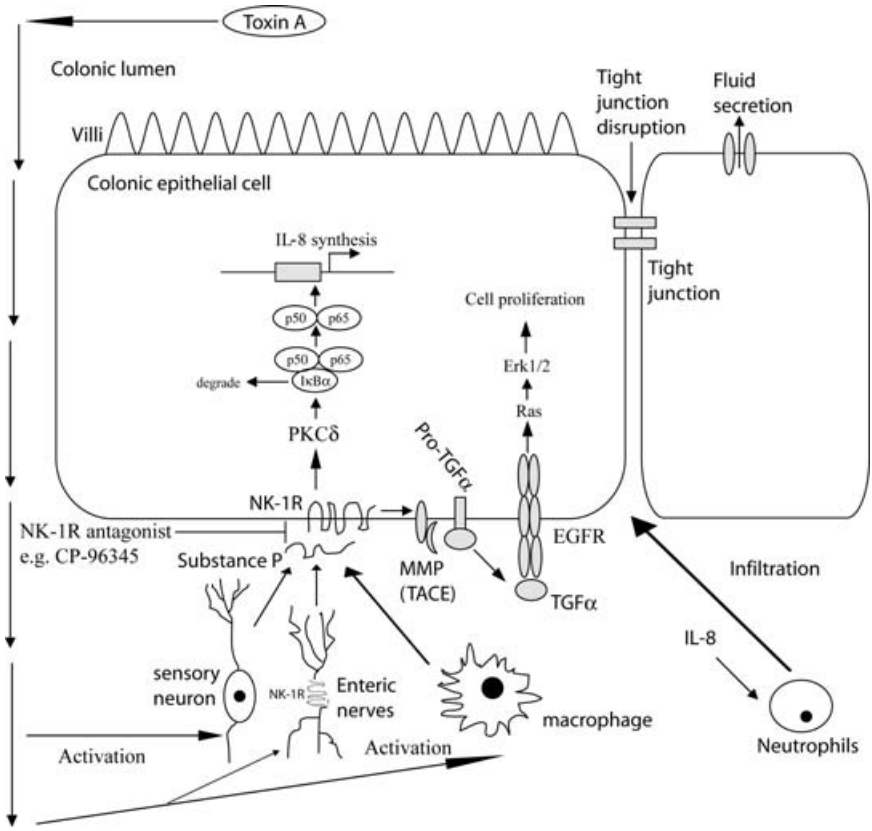

FIGURE 1. Substance P–dependent neuroimmune signaling during *C. difficile* toxin A–induced intestinal inflammation and tissue repair. Toxin A or other pathological stimuli activates substance P (SP) synthesis from sensory neurons and/or macrophages. SP binds to its high-affinity neurokinin-1 receptor (NK-1R) in target cells and activates protein kinase C δ phosphorylation, which activates the NF-κB system, leading to increased synthesis of NF-κB-driven proinflammatory genes, such as interleukin-8 (IL-8). IL-8 and other potent chemoattractants released from epithelial and lamina propria cells cause neutrophil infiltration and release of potent neutrophil mediators. Enteric nerves also express NK-1R as well as SP. During the repair phase of intestinal inflammation, SP binds to NK-1R, and induces matrix metalloproteinase activity that releases TGF-α into the external environment. TGFα binds to epidermal growth factor receptor, which activates ERK and mediates cell proliferation participating in tissue recovery.

SP AND NK-1R IN IMMUNE CELLS: REGULATION DURING AN INFLAMMATORY RESPONSE

Although SP and its receptors have been primarily associated with neurons, both centrally and peripherally, several pieces of evidence indicate that

immune cells in several different organs, including the gastrointestinal tract, express SP, or its NK-1 receptor, or both. Thus, SP has been localized in human dendritic cells,[23,24] brain microglia,[25] mononuclear phagocytes,[26] and lymphocytes.[27,28] These immune cells may have SP-related autocrine regulatory pathways as well as influencing other cells containing SP receptors in a paracrine manner. Along these lines, NK-1R is found in T lymphocytes,[29] B lymphocytes,[30] macrophages,[26] dendritic cells,[24] neutrophils,[31] mast cells,[32] and natural killer cells.[33]

Accumulating evidence indicates that the SP-NK-1 receptor system represents a major immunoregulatory circuit involved in several physiological and pathophysiological gut responses and disease states. In the gut, lamina propria macrophages (LPMs) express both SP and NK-1R,[34] while T cells express a functional NK-1R.[35] SP-NK-1 receptor neuroimmune interactions participate in basic colonic responses, such as chloride secretion, gut permeability, and modulation of inflammation. This review will discuss the available evidence for participation of SP-mediated responses in several disease states, including inflammatory bowel disease, enterotoxin-mediated diarrhea and inflammation, and infectious diarrhea of various etiologies. We summarize here evidence showing the importance of SP-NK-1 receptor neuroimmune interactions in intestinal inflammation and we highlight the signaling mechanisms involved in this response. We also attempt to provide information that points to putative new therapeutic approaches in intestinal inflammatory and secretory states where SP appears to play an important role.

ROLE OF SUBSTANCE P IN *CLOSTRIDIUM DIFFICILE*–INDUCED DIARRHEA AND INFLAMMATION

Clostridium difficile is the primary etiologic agent of antibiotic-associated colitis in animals and humans and is an emerging health problem in hospitalized patients in the USA and abroad.[36] *Clostridium difficile* mediates its intestinal effects by releasing two exotoxins, toxins A and B, that bind to colonocytes and initiate a diarrheal response characterized by increased intestinal permeability, destruction of colonocytes, activation of immune cells of the lamia propria, and release of proinflammatory cytokines leading to activation and transmigration of neutrophils.[37] The mechanisms of *C. difficile* toxins can be divided into direct effects on enterocytes and colonocytes and indirect effects on subepithelial cells triggered by cytokines, neuropeptides, and other neuroimmune mediators.

One important characteristic of *C. difficile* toxin A pathophysiology is the dependency of toxin A–associated intestinal secretion and inflammation on activation of intestinal nerves and neuropeptides, and in particular SP. Experiments using anesthetized animals demonstrated that enteric nerves and capsaicin-sensitive sensory neurons mediate toxin A-induced ileal fluid secretion,

mucosal permeability, mast cell degranulation, and intestinal inflammation.[38–40] Pretreatment with the capsaicin vanilloid receptor subtype 1 (VR1) antagonist, capsazepine, also significantly inhibited toxin A–induced colitis,[41] indicating a neurogenic inflammatory circuit that signals sensory neurons in the spinal cord. Because SP is the major constituent of primary sensory neurons, our laboratory and that of others examined the possibility that SP is involved in the intestinal effects of toxin A. Parenteral administration of a nonpeptide NK-1 receptor antagonist in rats inhibited all toxin A–associated secretory and inflammatory responses,[39,42] suggesting a proinflammatory role for SP in this enterotoxin model of intestinal inflammation. Toxin A also induces an early (30 min) increase of SP in the cell bodies of dorsal root ganglia followed by increased SP expression in the intestinal mucosa[34] which initiates or propagates the intestinal inflammatory response. In parallel, toxin A administration also stimulates a prompt upregulation in NK-1R expression in the intestinal mucosa, an effect also evident in mucosal biopsies of patients with *C. difficile* colitis.[43] A major role for SP and its NK-1 receptor in the mediation of intestinal inflammation in the toxin A colitis model was directly confirmed by studies demonstrating that mice genetically lacking NK-1 receptors have significantly attenuated intestinal responses to toxin A, including tumor necrosis factor α (TNF-α) expression.[44] *In vivo* evidence also indicates that the peptides neurotensin and corticotropin-releasing hormone, both of which play a proinflammatory role in the ileal loop model of toxin A colitis, mediate their effect, at least in part, by release of SP in the intestinal mucosa.[45,46]

The cell surface enzyme called neutral endopeptidase (NEP) is responsible for degrading SP in extracellular fluid and terminating its proinflammatory effects.[47] The importance of the SP/NEP system was also confirmed in the *C. difficile* toxin A enteritis model. Thus, compared to the wild-type, NEP-deficient mice had exacerbated inflammatory and secretory responses, while pretreatment of recombinant NEP prevented exacerbated inflammation in response to toxin A.[48] In contrast, pretreatment of wild-type mice with the NEP inhibitor phosphoramidon exacerbated toxin A enteritis.[48] Thus, NEP can terminate enteritis induced by toxin A by degrading SP.

SP–Mast Cell Interactions in the Toxin A Model

Several studies indicate that nerve mast cell communication participates in the pathophysiology of intestinal inflammation,[49] and neuronal SP interacts with mast cells in the intestinal mucosa.[50] Early studies indicated that injection of toxin A into ileal loops caused mast cell degranulation, and stimulated release of rat mast cell protease II (RMCPII),[38,51] a specific mucosal mast cell protease. Interestingly, ablation of sensory neurons with capsaicin or administration of the NK-1R antagonist CP-96345 dramatically reduced release of RMCPII upon exposure to toxin A (TxA),[38] indicating SP–mucosal mast cell

interactions during toxin A-induced neurogenic gut inflammation. The role of mast cell–SP communication in the development of intestinal inflammation was further confirmed in the toxin A model by use of mast cell–deficient mice and NK-1R antagonists. Results from these studies indicated SP-mast cell–dependent pathways in the regulation of toxin A–induced secretion and neutrophil infiltration.[52] Whether SP can directly stimulate proinflammatory responses in intestinal mast cells and whether mucosal mast cells express SP receptors is still a matter of controversy. Part of this controversy appears to be the difficulty in isolating pure gut mast cell preparations retaining full function. Early studies indicated that human mucosal mast cells isolated from the intestine respond to supraphysiological concentration of SP (10^{-4} M) by releasing histamine.[32] Recent results indicated that, while nonactivated human mast cells do not respond and do not express NK-1R, they do so upon IgE stimulation.[53]

Intestinal Monocytes/Macrophages and SP Responses During Toxin A Enteritis

Macrophages are implicated in the pathophysiology of intestinal inflammation and IBD.[54] SP can also stimulate IL-1β production from human blood monocytes,[55] and activated monocytes have enhanced responses to SP.[56] Rat peritoneal macrophages express low levels of SP and NK-1R mRNAs which can be substantially increased after LPS exposure.[57] Evidence also indicate that expression of NK-1R expression by macrophages can be increased by IL-4 and IFN-γ, suggesting a T cell macrophage communication that might involve SP and NK-1R.[58] SP-NK-1R interactions at the intestinal macrophage level might also modulate intestinal inflammation. Castagliuolo *et al.* indicate that LPMs isolated from toxin A-injected loops release large amounts of TNF-α and SP, compared to control LPMs. Moreover, pretreatment of rats with a NK-1R antagonist inhibited toxin A–mediated TNF-α release from isolated LPMs, while LPMs obtained from toxin A–exposed intestine incubated *in vitro* with SP showed enhanced TNF-α secretion compared to control LPMs, which did not respond to SP.[34] In addition, incubation of activated LPMs with the NK-1R antagonist CP-96345 showed diminished TNF-α release.[34] Thus, *in vivo* activated LPMs secrete SP during an intestinal inflammatory response, which leads to increased cytokine production, pointing to an autocrine/paracrine regulation of cytokine secretion by SP during intestinal inflammation.

ROLE OF SP AND NK-1R IN IMMUNE RESPONSES DURING *SALMONELLA* INFECTION

Salmonella gastroenteritis is an important foodborne infection associated with significant morbidity around the world. Studies in animal models indicate

that SP participates in the pathophysiology of *Salmonella* infection. Thus, SP exposure to a strain of *Salmonella* inhibited binding to lymphocytes, with a more pronounced effect on the T-suppressor/cytotoxic T-cell subset.[59] Oral intake of *Salmonella* in mice promptly results in substantial NK-1R upregulation in the Peyer's patches and mesenteric lymph nodes.[60] In murine salmonellosis, administration of a NK-1R antagonist prior to *Salmonella* resulted in an earlier onset of infection, increased mortality, and reduced mucosal IL-12 and IFN-γ mRNA levels in infected mice.[60] Moreover, IL-12 protects mice from *Salmonella* infection,[61] and SP via its NK-1 receptor stimulates IL-12 release from macrophages.[62] Thus, on the basis of this evidence, SP and NK-1R might play an important role in protecting immune responses in *Salmonella* gastroenteritis, via macrophage-dependent responses. A recent study by Walters and colleagues using NK-1R-deficient mice, however, projects a different view. These studies showed that oral immunization of NK1R KO mice with a *Salmonella*-CFA/I vaccine resulted in elevated mucosal and systemic IgA responses to CFA/I fimbriae associated with increased IL-5- and IL-6-producing $CD4^+$ Th2 cell populations.[63] Moreover, there were no differences in the ability of these vaccines to protect mice between NK-1R KO and wild-type mice. However, innate resistance to wild-type *Salmonella* was significantly enhanced in NK-1R-deficient mice, suggesting diminished proinflammatory responses in the absence of SP/NK-1R system.[63] Despite the different results, however, it is clearly evident that SP and its NK-1R contributes to intestinal immunity during *Salmonella* infection.

SP-DEPENDENT NEUROIMMUNE INTERACTIONS IN PARASITIC INFECTIONS

Trichinella spiralis

Trichinella spiralis is a helminthic parasite affecting both animals and humans, whose pathophysiology involves extensive neuroimmune interactions. Mast cells and Th2 cells play an important role in the development of *T. spiralis* infection and several of its intestinal responses.[64,65] For example, increased levels of substance P are evident in the myenteric plexus of *T. spiralis*-infected rats,[66] although in the guinea pig and ferret intestine lower intestinal levels were noted.[66,67] Pretreatment with either a SP antibody or the NK-1R antagonist CP 96345 effectively diminished inflammatory responses in the jejunum of *T. spiralis*–infected mice.[68,69] Moreover, in the inflamed intestine of rats infected with *T. spiralis*, activity of the SP-limiting enzyme NEP is significantly downregulated, leading to reduced SP degradation.[70] Together, these results indicate that SP-NK-1R-dependent mechanisms might regulate intestinal inflammatory responses during this parasitic infection.

Nippostrongylus brasiliensis

Animal models of *N. brasiliensis* infection have been extensively used to study intestinal pathophysiology, including inflammation and permeability-related responses, where neuronal mast cell interactions appear to play an important role. In a rat *N. brasiliensis* model, the majority of intestinal mucosal mast cells were in contact with nerves in the small intestinal submucosa, including SP-containing nerves,[49,71] providing anatomical evidence for cross-talk between the immune and nervous systems in the gut. Ablation of extrinsic sensory neurons with capsaicin worsened intestinal inflammation in *N. brasiliensis*–infected rats, without affecting the duration of the infection.[72] Moreover, tissue from *N. brasiliensis*–infected rats contained increased amounts of immunoreactive SP immunoreactivity, primarily on nerve fibers.[73] Thus, SP-containing sensory neurons may play a protective role in the development of *N. brasiliensis* infection and SP–mast cell interactions might participate in this response.

Schistosoma mansoni

Schistostoma mansoni infections represent an important clinical condition affecting the intestine, the liver, and the spleen, characterized by formation of granulomas containing several immune cells.[74] Several pieces of evidence demonstrate extensive cross-talk between neuropeptides, including SP and NK-1R, and immune cells affecting the pathophysiology of schistosomiasis.[74] Thus, eosinophils from schistosoma granulomas express SP at the protein and mRNA level,[75] and NK-1R is evident in T lymphocytes from these ganulomas.[76] SP modulates immunoglobulin secretion in granuloma cells isolated from infected mice,[77] and stimulates IFN-γ secretion from primed granuloma cells.[78] SP mRNA is also detectable in lamina propria and spleen macrophages isolated from schistosome granulomas.[79] Moreover, granuloma macrophages from STAT6-deficient mice had several-fold higher SP mRNA expression, while, in contrast, STAT4 knockout mice had diminished SP mRNA expression in the same cell population.[79] Along these lines, IL-12, which signals via STAT4 to induce Th1-type inflammation, induced SP mRNA expression in macrophages from *Schistosoma*-infected mice and lamina propria mononuclear cells.[79] Thus, SP mRNA, expressed in macrophages during inflammatory responses, is regulated by IL-12 and STAT4-dependent signaling. The importance of SP and its receptor in the development of schistosomiasis granulomas is underscored by studies using NK-1R antagonists and mice deficient in NK-1R 1 receptor.[78,80] Preprotachykinin C mRNA and HK were also found in schistosoma granuloma T cells and macrophages and both SP and HK stimulated IFN-γ production, while a NK-1R antagonist inhibited this response.[81] Thus, it is quite evident that HK and SP are expressed at sites of chronic inflammation, and their expression is regulated during an inflammatory response during this helminthic infection. Moreover, SP and NK-1R immunomodulation

and proinflammatory responses are also subject to regulation by another peptide, somatostatin, acting via its type 2 (SSR2) receptor.[82]

Inflammatory Bowel Disease

Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), is a group of chronic debilitating diseases with substantial morbidity and mortality affecting millions of patients worldwide. Although the etiology of IBD remains under investigation, several studies point to an important role for SP and its receptors in the pathophysiology of this disease. Early studies indicated increased expression of SP receptor binding sites in IBD patients expressed in the submucosa, muscularis mucosa and external circular, and longitudinal muscle.[10] Patients with CD showed increased NK-1R receptors in lymphoid aggregates, small blood vessels, and enteric neurons, while in UC patients, these receptors were only evident in lymphoid aggregates and small blood vessels, but not in enteric nerves.[83,84] Later studies confirmed increased NK-1R expression in the intestine of these patients and indicated that lamina propria mononuclear cells, as well as epithelial cells also express NK-1R.[12,14] Raithel *et al.* also indicated that colonic mucosal explants from IBD patients showed enhanced mucosal mast cell mediator secretion in response to SP,[85] suggesting a functional role for these receptors in IBD. While some studies found increased SP expression in IBD tissues,[86–88] other studies failed to demonstrate such a response.[89,90]

Diarrhea represents one of the predominant symptoms in IBD, and SP–NK-1R interaction appears to participate in this response. Electrophysiologic studies with animal small intestine and colon indicate the ability of SP to mediate intestinal secretion in normal intestine.[91–94,95] These studies also demonstrated that enteric nerves and mast cells might participate in these SP-mediated responses. Experiments with human colonic strips mounted in Ussing chambers indicate that SP, via NK-1R, is able to stimulate chloride secretion in human intestine via mast cell and nerve-dependent mechanisms.[13] These results suggest extensive neuroimmune modulation of NK-1R-mediated secretion in human colon. Moreover, Riegler *et al.* also showed that SP caused histamine and prostaglandin release from human colonic mucosa, while histamine and prostaglandin inhibitors reduced the secretory response to SP.[13] The pathophysiologic importance of the NK-1R diarrheal response was confirmed by Turvill *et al.*, demonstrating that the cholera toxin–mediated intestinal secretion involves NK-1 and NK-2 receptors.[96]

SP AND ANIMAL MODELS OF INFLAMMATORY BOWEL DISEASE

Apart from indirect evidence from human studies, results from animal models of IBD strongly suggest a functional role for SP receptors in the pathophysiology of this disease. For example, administration of a NK-1 receptor

antagonist to rats reduced the severity of colitis and alterations of contractility 14 days after intracolonic administration of trinitrobenzene sulfonic acid (TNBS), an animal model resembling CD.[97] Genetically engineered NK-1R-deficient mice were also protected from acute colitis 2 days after intracolonic TNBS administration.[98] Moreover, injection of an NK-1R antagonist to rats also reduces colonic inflammation and oxidative stress in dextran sulfate–induced colitis, a model resembling UC.[99] Ileal pouch–anal anastomosis (IPAA) is a frequent surgical option for patients operated on for chronic UC requiring colectomy. However, this anastomosis is often associated with ileal pouch inflammation and this effect can be recapitulated in rats with experimental IPAA. Interestingly, administration of an NK-1R antagonist to rats with IPAA was effective in reducing inflammatory responses in the ileal pouch,[100] indicating that SP receptor antagonism might be a therapeutic option in clinical pouchitis. IL-10-deficient mice develop spontaneous colitis characterized by a Th1-driven response and have been used as model to study IBD. Administration of non-steroidal anti-inflammatory compounds (NSAIDs) to young IL-10 mice results promptly in dramatic ileitis and colitis,[101] characterized by the appearance of NK-1R in mucosal T cells regulated by an interplay between IL-12 and IL-10.[35] Moreover, NK-1R antagonism in these mice alters intestinal inflammation, confirming the importance of SP and its receptor in the development of intestinal inflammation.[35]

As discussed above, NEP is a SP-degrading enzyme that limits availability of this peptide during an inflammatory response. NEP knockout mice have substantially elevated SP colonic levels and increased colonic permeability under basal conditions than wild-type mice.[102] The severity of TNBS-induced colitis in NEP knockout mice was also substantially worsened compared to the wild-type, while this effect was prevented by recombinant NEP and NK-1R antagonism.[102] Thus, increased bioavailability of SP due to lack of the SP degrading enzyme NEP leads to increased colonic inflammation.

Mechanisms of the Proinflammatory Effects of SP

Many of the studies outlined above clearly indicate that SP, acting via NK-1R, plays an important role in the pathogenesis of intestinal inflammation. The mechanism of SP–NK-1R participation in intestinal inflammation involves release of inflammatory mediators because SP directly stimulates cytokine production such as IL-1β, IL-6, IL-8, and TNF-α from several diverse cell types.[34,55,103–105] Transcription of proinflammatory genes by SP–NK-1R interactions involves NK-1R-dependent activation of the inflammatory transcription factor NF-κB in target cells.[15,106,107] SP-induced NF-κB activation and cytokine gene transcription also involve the Rho family of small molecular weight GTPases, RhoA, Rac1, and cdc42,[15] and can be dependent[15,107]

or independent of MAP kinase activation. Recent evidence also indicates that SP induces phosphorylation of protein kinase C, including the delta, theta, and epsilon isoforms[108] in human colonocytes. These studies also showed that SP-induced PKC delta activation is functionally involved in NK-1R-mediated NF-κB activation and IL-8 secretion in response to SP.[108]

As discussed above, NK-1R expression is increased in several models of intestinal inflammation. Because cytokine levels are also increased during colitis, cytokines can, in turn, affect expression of NK-1R. Consistent with this hypothesis, Simeonidis *et al.* demonstrated that exposure of human monocytic THP-1 cells expressing authentic NK-1R to IL-1β and TNF-α stimulated increased NK-1R gene expression at the mRNA and protein levels.[109] Moreover, NK-1R expression in response to cytokine stimulation was diminished by transfection of THP-1 cells with the NF-κB inhibitor IκBα, indicating that this transcription factor is tightly involved in regulation of NK-1R gene expression during an inflammatory response. Along these lines, IL-12 and IL-18 induce T cells to express NK-1R through NF-κB activation, while IL-10 inhibits this response.[110] Similar NF-κB-dependent regulation of NK-1R expression has also been reported in astrocytes,[111] and human alveolar macrophages,[112] while Reed *et al.*[113] demonstrated that NF-κB activation precedes NK-1R expression in experimental colitis, providing a functional correlate to this response. Taken together, these results indicate that the proinflammatory factor NF-κB is involved in both SP proinflammatory signaling and regulation of the SP receptor NK-1 during colitis.

SP MEDIATES TISSUE RECOVERY VIA EPIDERMAL GROWTH FACTOR RECEPTOR ACTIVATION

Besides acting as a pro-inflammatory mediator, SP can also induce cell proliferation in several cell types such as T lymphocytes,[29] skin fibroblasts,[114] and smooth muscle cells.[114] Castagliuolo *et al.*[98] reported that mice lacking NK-1R had significantly worsened colitis in the chronic phase of both the DSS and TNBS colitis models, indicating that SP can also promote mucosal healing during an inflammatory response. Further experiments indicated that this effect is likely mediated by SP-induced cell proliferation via a communication between the NK-1R and the epidermal growth factor receptor (EGFR) as shown in colonic fibroblasts *in vivo*, human astrocytoma cells, as well as human colonic epithelial cells.[98,115,116] SP-induced transactivation and tyrosine phosphorylation of EGFR, leading to cell proliferation, involves the formation of activated EGFR complex with adapter proteins SHC and Grb2.[115] Moreover, in human colonocytes, NK-1R-induced EGFR and MAPK activation and cell proliferation involves release of matrix metalloproteinases (most likely TACE) and secretion of transforming growth factor (TGF-α), signaling mechanisms likely to be involved in the protective effects of NK-1R in chronic colitis.

SUMMARY AND THERAPEUTIC IMPLICATIONS

As we discussed in this review, substantial evidence from *in vitro* and *in vivo* approaches, as well as evaluation of responses in human colon with IBD, suggest that SP is an important mediator of the neuroimmune response related to several disease states. These interactions are important in the initiation and progress of inflammatory processes as well as in several symptoms related to inflammatory diarrhea of diverse etiologies. The ability of proinflammatory cytokines to modulate expression of SP receptors on neuronal, immune, and epithelial cells, together with increased expression of SP itself in intestinal inflammation, suggests that these molecules may represent a potential therapeutic target for treatment of several intestinal inflammatory states. The development of highly specific neurokinin-1 receptor antagonists by several major pharmaceutical companies and their current use in different clinical conditions, such as depression and anxiety, rheumatoid arthritis, chemotherapy- or radiotherapy-induced emesis, among others, opens up the possibility for their use in intestinal inflammation and IBD. Clinical trials in humans assessing the utility of NK-1R antagonists for the treatment of IBD are limited, and the results of a pilot study have not been reported.

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