DOI: 10.1002/JLB.3MIR0817-348R

JLB JOURNAL OF LEUKOCYTE BIOLOGY

REVIEW

Truncation of neurokinin-1 receptor—Negative regulation of substance P signaling

Sergei Spitsin[1] | **Vasiliki Pappa**[1] | **Steven D. Douglas**[1,2]

[1]Department of Pediatrics, Division of Allergy and Immunology, Children's Hospital of Philadelphia Research Institute, Philadelphia, Pennsylvania, USA

[2]Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA

Correspondence
Steven D. Douglas, Children's Hospital of Philadelphia, 3615 Civic Center Blvd. Abramson Research Center, Room 1208A, Philadelphia, PA 19104, USA.
Email: douglas@email.chop.edu

Abstract

Substance P (SP) is a tachykinin peptide, which triggers intracellular signaling in the nervous and immune systems, as well as, other local and systemic events. The interaction between SP and its receptor, neurokinin-1 receptor (NK1R), results in major downstream cellular actions, which include changes in calcium fluxes, ERK, and p21-activated kinase phosphorylation and NFκB activation. Two naturally occurring variants of the NK1R, the full-length, 407 aa receptor (NK1R-F) and the truncated, 311 aa isoform (NK1R-T), mediate the actions of SP. Receptor truncation partially disrupts signaling motifs of the carboxyl tail, a critical site for mediating NK1R signaling, resulting in a "less-efficient" receptor. Although NK1R-F is the predominant isoform in the central and peripheral nervous systems, NK1R-T is expressed in several tissues and cells, which include monocytes, NK cells, and T-cells. The SP binding domain is not affected by truncation and this site is identical in both NK1R receptor isoforms. However, while cells expressing NK1R-F respond to nanomolar concentrations of SP, monocyte and macrophage activation, mediated through NK1R-T, requires micromolar concentrations of SP in order to elicit signaling responses. Elevated plasma levels of SP are associated with increased inflammatory responses and NK1R antagonists reduce inflammation and cytokine production *in vivo*. This mini review presents and discusses the novel hypothesis that the expression of NK1R-T on immune system cells prevents immune activation in a milieu, which usually contains low concentrations of SP and, thus, maintains immune homeostasis. In contrast, in the activated neuronal microenvironment, when SP levels reach the threshold at tissue sites, SP promotes immune activation and modulates monocyte/macrophage polarization.

KEYWORDS

inflammation, macrophages, monocytes, neurokinin-1 receptor, neurokinin-1 receptor antagonists, substance P, tachykinins

1 | INTRODUCTION

The neurokinin-1 receptor (NK1R) is a tachykinin receptor that regulates a variety of physiologic and pathophysiologic pathways, including immune system functions. The receptor was first isolated in 1989 in rat tissues[1] and subsequently, in human cells and cells from several other species.[2] In human cells, with some minor exceptions, the NK1R is ubiquitous and is constitutively expressed.[3] Although NK1R binds to several tachykinins, including neurokinin A and B, it has the highest affinity for the neuropeptide substance P (SP), which is secreted by many cell types.[4,5]

NK1R is a member of the class A, "rhodopsin-like," G protein-coupled receptor (GPCR) family. These receptors are characterized by an extracellular N-terminal region, 7 transmembrane alpha helices connected by 3 alternating intracellular and extracellular loops, and a C-terminal intracellular tail. In NK1R, a disulfide bond connects the first 2 extracellular loops.[4] In human cells, SP binds to the N-terminus, the second transmembrane domain, and the second and third extracellular loops of the NK1R.[2] In contrast, the C-terminal tail binds to the α-subunit of heterotrimeric G proteins, as well as other signaling molecules, which include β-arrestin and GPCR kinases.[4,6]

GPCRs are one of the largest families of cell-membrane receptors. This superfamily of proteins is phenotypically diverse, which

Abbreviations: GPCR, G protein-coupled receptor; MDMs, monocyte-derived macrophages; mGluR, metabotropic glutamate receptor; NK1R, neurokinin-1 receptor; NK1R-F, neurokinin-1 receptor, full length; NK1R-T, neurokinin-1 receptor, truncated; PKC, protein kinase C; PMA, phorbol myristate acetate; SP, substance P; TACR1, tachykinin receptor 1

Received: 31 August 2017 | Revised: 22 November 2017 | Accepted: 7 December 2017

is attributable to genetic variation, posttranslational modifications, and alternative splicing. In the majority of GPCRs, alternative splicing affects the C-terminal tail, which has a dramatic effect on signaling, regulation, and functional activity.[6,7] NK1R has a naturally occurring truncated C-terminal isoform, NK1R-T. Although truncation does not directly affect the SP binding site, there is evidence that NK1R-T has decreased ligand binding affinity, as well as, alternate tissue expression, signaling and regulatory properties in comparison with the NK1R-F.[4]

NK1R-T is expressed in immune cells, and therefore NK1R alternative splicing has a profound effect on immune signaling modulation. NK1R signaling participates in a plethora of diseases, which range from infection to cancer, and there is recognition of an emerging role for NK1R-T effects in disease pathophysiologic mechanisms.[8–12] The investigations of NK1R-T regulation and signaling are an important component toward the investigation of pathogenesis models and the determination of the potential therapeutic utility of NK1R inhibition. Our previous review, Douglas and Leeman[4] addressed the overall role of NK1R in the immune system, in infections and in inflammation. In the current review, we address the specific hypothesis of negative regulation of SP signaling through NK1R-T.

2 | THE TACHYKININ—SP

SP is an 11 aa undecapeptide. It is a member of the tachykinin superfamily, which contains over 40 peptides, including neurokinin A, neurokinin B, and neuropeptide-γ. SP signaling has ancient evolutionary roots. For example, SP promotes neoblast proliferation and migration in planarians indicating an early evolutionary neural regulation of stem cell biology.[13] A functional SP pathway has also been described in insects[14,15] and recently in fish.[16]

SP was initially identified in 1931 by von Euler and Gaddum,[17] as a compound that causes *ex vivo* hypotension in rabbits, as well as contraction of the rabbit jejunum. SP was termed a "tachykinin" as it produced a rapid contractile response in smooth muscle. It was first isolated from extracts of bovine brain tissue by Chang and Leeman in 1970[18] and subsequently sequenced by Chang et al. in 1971.[19] Since this initial seminal discovery, by Leeman and her colleagues, SP expression has been identified in different organs and cell systems, including the central and peripheral nervous systems, as well as the immune system.[20–23]

In the nervous system, SP acts as a neurotransmitter. It is released from neurons in both the peripheral and CNS, as well as from non-neuronal cells, such as inflammatory and endothelial cells.[21,24] In the CNS, SP is commonly colocalized with other neurotransmitters including serotonin, dopamine, GABA, acetylcholine and corticotropin-releasing hormone. Thus, SP is involved in complex brain functions, which include neuronal sensory transmission associated with pain, depression, anxiety, and central responses to stress.[20,23,25,26] In the immune system, SP has pro-inflammatory effects.[27] In some cases, SP augments inflammatory responses in the respiratory and gastrointestinal tract, as well as the CNS, most likely, by affecting monocyte/macrophage polarization.[22,23,28–39]

3 | NK1R ALTERNATIVE SPLICING AND ITS ISOFORMS

SP preferentially binds to the NK1R. This peptide–protein interaction is a central mediator in both immune and nervous system signaling.[4,40] Two isoforms of NK1R have been identified in human and animal tissues, as a result of alternative splicing: a full-length isoform (NK1R-F), consisting of 407 aa, and a truncated isoform (NK1R-T), consisting of 311 aa.[4,41,42] The first characterization of the truncated and full-length forms was performed by Boyd et al.[42] using photoaffinity labeling in rat submaxillary glands. In mammals, the NK1R-F receptors identified so far are 407 aa long and have over 94% homology with each other.[2,43] The NK1R-T isoform has also been identified in several species including rats, mice, and guinea pigs.[43] Additionally, in *Bufo marinus*, 3 different splice variants of NK1R have been described, which contain 390, 371, and 309 aa, respectively.[44]

NK1R is encoded by the *TACR1* gene, which, in humans, is 1,221 nucleotides long and consists of 5 exons.[45] The NK1R-T isoform is produced by retention of the fourth intron in the mature mRNA transcript resulting in the introduction of a premature stop codon at the end of exon 4 and before the start of exon 5 (Fig. 1).[4] Intron retention is a relatively infrequent occurrence in human cells and it is associated with shorter introns or weaker splice sites.[46] The triggers and regulatory mechanisms of NK1R alternative splicing remain unknown. However, in other GPCRs, splicing regulates intracellular signaling, agonist binding, receptor internalization, and tissue-specific protein expression.[47] Indeed, NK1R isoforms have distinct tissue distribution with NK1R-F being predominately expressed in the CNS, whereas NK1R-T has higher expression in peripheral tissues.[48,49]

The 2 human NK1R splice variants have been cloned and expressed, and several functional differences between them have been observed (for review see Refs. 4,40). Studies performed using NK1R-T provide evidence that, although truncation does not directly affect the SP binding, the loss of the C-terminal tail reduces SP binding affinity by 10-fold.[41] A similar phenomenon has been observed in the metabotropic glutamate receptor (mGluR), where reduced agonist potency occurs in the mGluR1b and mGluR1c, truncated C-tail isoforms.[50] Because truncation of the C-terminal tail affects interaction with β-arrestin, the mGluR1b and mGluR1c isoforms exhibited altered internalization and desensitization properties.[51] Similar effects occur in the prostaglandin EP3 receptor isoforms that were identical up to the seventh transmembrane domain but had differences in the C-terminal tail.[52] Leeman was the first investigator to predict that the absence of the C-terminal phosphorylation site will lead to impairment of NK1R desensitization.[42] In NK1R-T, truncation of the C-terminal tail decreases internalization and affects desensitization.[53,54] Additionally, in the EP3 isoforms, constitutive activity inversely correlates with the length of the C-terminal tail.[52] Similarly, constitutively

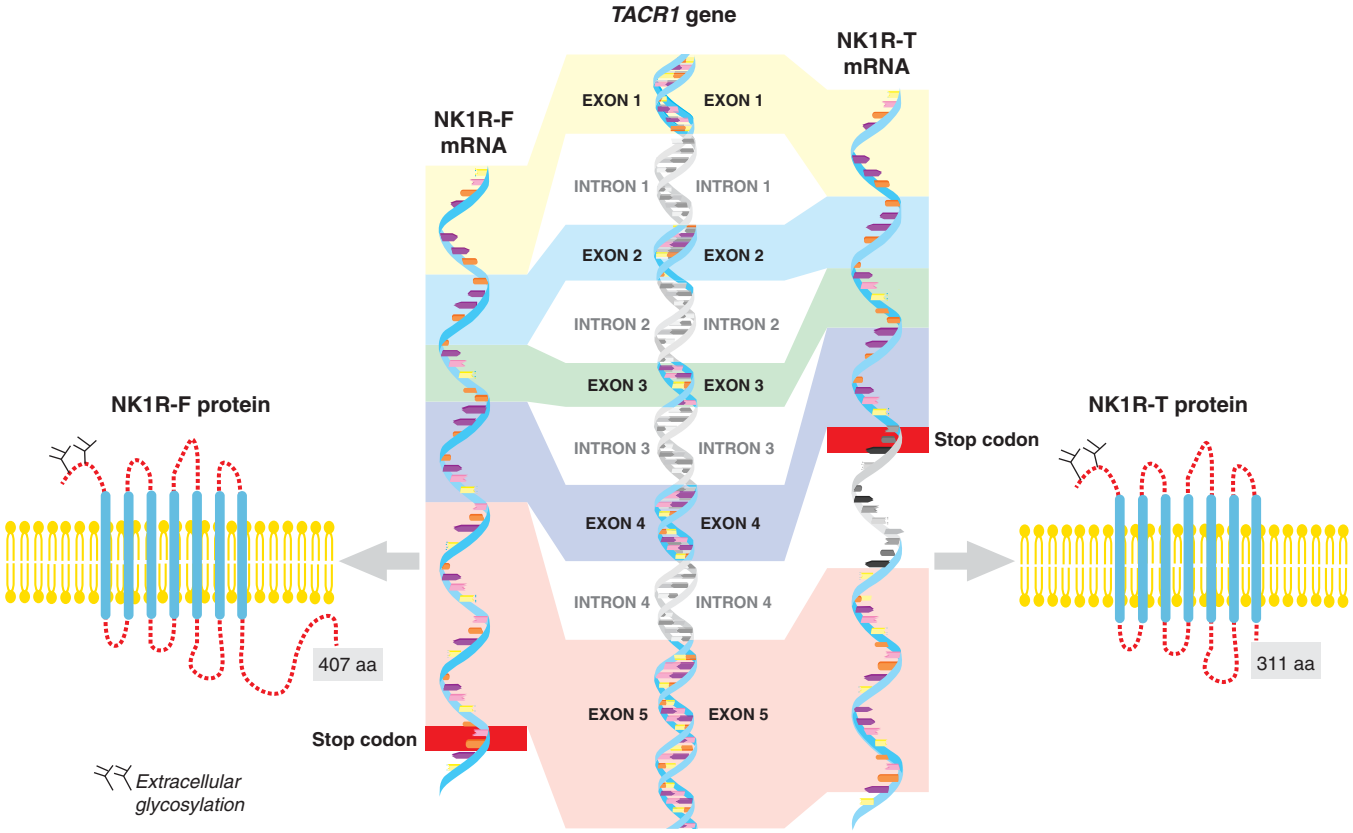

FIGURE 1 NK1R alternative splicing generates 2 receptor isoforms. The *TACR1* gene contains 5 exons and 4 introns. During transcription it can produce either a canonical (left) or an alternative (right) mRNA splice variant. The canonical NK1R mRNA transcript contains only the 5 exons from the *TACR1* gene and it is translated into the full-length receptor, which is a protein 407 aa long. In the alternative mRNA transcript, intron 4 is retained. As a result, during translation the stop codon is introduced after the fourth exon, which produces a truncated, 311aa long receptor

active forms of NK1R have been documented in glioblastoma cell lines.[55]

4 | FUNCTIONAL DIFFERENCES BETWEEN NK1R ISOFORMS

Truncation of the 96 aa terminal tail of NK1R results in several functional differences between the 2 isoforms (Fig. 2 and Table 1). The carboxyl tail of NK1R, along with the third intracellular loop, contains sites that are critical for signaling.[53,54,56] Overall, in NK1R, the intracellular loops interact with G-proteins, whereas the extracellular loops are the sites of SP binding.[57–59] Moreover, the intracellular C-terminal tail contains phosphorylation sites and mediates the internalization of the receptor following activation.[60–62] The C-terminal tail of NK1R is also required for receptor desensitization. Although desensitization is linked to phosphorylation,[60,61] it is uncertain whether it is directly linked to receptor internalization.[60] An artificially truncated NK1R, which lacks the last 82 C-terminal aa residues (324 aa receptor) and displays regular G-protein mediated cell signaling properties, is resistant to desensitization.[54]

In NK1R-T, the carboxyl tail signaling motifs are partially disrupted, resulting in a receptor that is "less efficient," in response to stimulation with its preferred ligand, SP. NK1R-F has a higher affinity for SP, as well as other agonists and antagonists.[41,63] Although similar to the majority of GPCR splice variants, the extracellular SP binding domain is unaltered and identical in both receptor isoforms.[6] Further, most studies of SP binding to the NK1R have been conducted with the full-length receptor with either agonist or antagonist using site-directed mutagenesis.[58] The available data are based on studies of cloned receptors transfected in artificial systems and have not yet been determined for primary cells.[6,41,60,61,63]

Our group and others have demonstrated that cells expressing NK1R-F, such as neurons and astrocytes, respond to nanomolar concentrations of SP. In contrast, we have demonstrated that monocytes/macrophages and lymphocytes require higher SP concentrations than cells expressing NK1R-F in order to produce maximal responses. Approximately 10 μM of SP[36,64–67] is required to release calcium from intracellular stores or to change cytokine and chemokine production in monocytes.[4,68,69] Micromolar SP concentrations may be outside of the physiologic range in the plasma, however, in the axonal microenvironment, SP may be present in higher concentrations. SP is difficult to measure *in situ* due to the very short half-life both *in vivo* and *ex vivo*.[70] In our investigations, 10 μM of SP added to tissue cultures of PBMC or macrophages decays to undetectable levels (<3 pM) after overnight incubation (data not shown). This observation is consistent with previous reports that SP rapidly degrades both *in vivo* and *in vitro*, with half-lives of less than 2 min[70] and 12 min,[71] respectively.

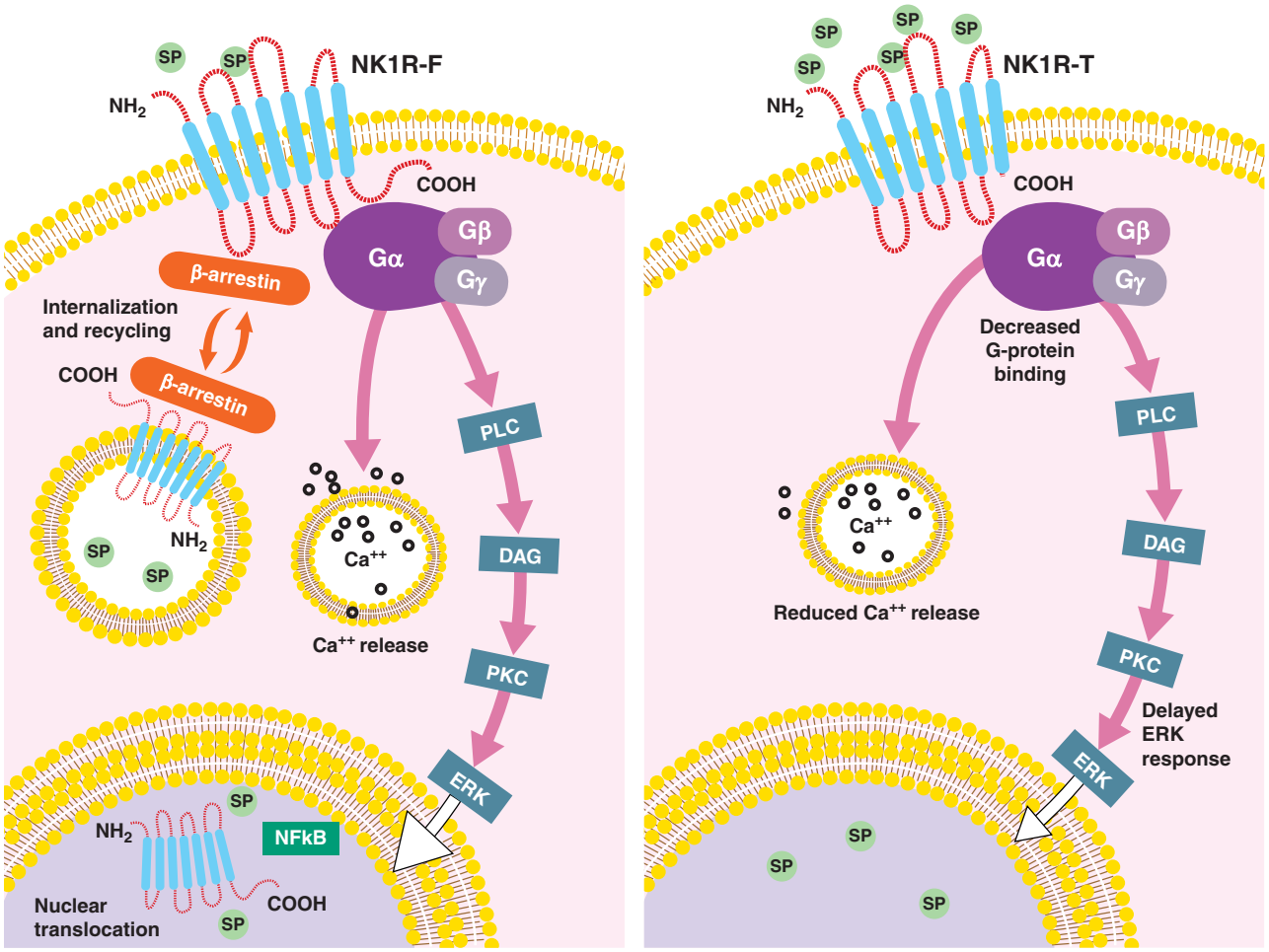

FIGURE 2 **The 2 NK1R isoforms are functionally different.** The truncation of the full-length NK1R leads to decreased SP binding affinity. The absence of the C-terminal tail impairs receptor internalization and recycling, resulting in a receptor resistant to desensitization. Because the intracellular C-terminal loop of the full-length NK1R (NK1R-F) is important for interactions with the G-proteins (Gα, Gβ, and Gγ), truncation affects the downstream activation of Phospholipase C (PLC) and the production of diacylglycerol (DAG). NK1R-T displays altered signaling properties with reduced Ca^{++} release, decreased Protein Kinase C (PKC) phosphorylation, and delayed ERK activation. The truncation of NK1R affects intracellular localization, and as a result, NK1R-T has decreased nuclear translocation

TABLE 1 Differences in functional properties of NK1R isoforms

Effect	NK1R-F	NK1R-T
Calcium mobilization	Yes; nanomolar SP[76]	Yes; micromolar SP[68]
PKCδ phosphorylation	Increased; nanomolar SP[76]	Decreased; only nanomolar SP tested[76]
Activation of ERK	Rapid[76]	Delayed[76]
NFkB activation	Yes[76]	No; only nanomolar SP tested[76]
Cytokine and chemokine production (including IL6, IL8, TNFα, MCP-1)	Yes; nanomolar SP[76]	Yes; micromolar SP[76,84]
Cell migration and chemotaxis	Direct; in cancer[33]	Indirect; up-regulation of chemokine and cross-talk between NK1R-T and CCR5[78]
NK cytotoxicity	Inhibition of contact-dependent cytotoxicity. The contribution of each individual isoform is unknown. NK cells express predominantly NK1R-T but a small amount of NK1R-F RNA has been detected.[67]	

Nevertheless, factors that may increase local SP concentrations include impulse releases of SP by neurons or the accumulation of SP along lipid membranes.[72–74]

There are functional differences between NK1R-F and NK1R-T, which require further investigation (Table 1). Initially, Douglas and others[4,40,67,75,76] reported that in comparison with NK1R-F, the interaction of NK1R-T with SP resulted in decreased PKCδ phosphorylation, slower ERK activation, lack of NFkB activation, lack of calcium mobilization, and decreased IL-8 mRNA expression. In these experiments, SP concentrations of 1 nM to 1 μM trigger the full-length

receptor but not the truncated NK1R.[76] However, in subsequent studies, SP triggered calcium mobilization in human monocytes and increased the production of several cytokines and chemokines, including IL-8, at concentrations in the 1–10 μM range. This finding demonstrates that NK1R-T is capable of mediating these responses in cellular environments that contain high concentrations of SP concentrations.[68] In the aforementioned experiments, the specificity of the SP effect on monocyte NK1R-T was confirmed with aprepitant, a specific NK1R antagonist.[68] In human clinical trials, aprepitant decreased plasma levels of IL-8 in HIV positive individuals.[77] NK1R-T mediates some signaling responses with low SP concentrations, SP concentrations as low as 100 nM, elicited enhanced CCL5-induced chemotaxis and ERK1/2 phosphorylation in monocytes.[78] These observations provide further support to our hypothesis that the absence of the NK1R C-terminal tail has a negative regulatory effect on NK1R signaling.

In the CNS, both NK1R-F and NK1R-T are abundant. The ratio between NK1R-F/T varies considerably between different anatomic sites in the brain and NK1R-F/T expression levels are affected by various pathophysiologic conditions.[48,79–83] Due to their functional differences, the expression of both isoforms is important to coordinate and regulate the functions that are mediated by SP/NK1R signaling.

5 | THE ROLE OF NK1R ISOFORMS IN THE IMMUNE RESPONSE

As was described earlier in this review, NK1R-F is the prevalent isoform in the central and peripheral nervous systems. Other tissues, however, including immune cells, predominantly express the "less efficient," NK1R-T isoform. Indeed, under most conditions, human monocytes, NK cells, and T-cells express NK1R-T.[4,48] SP/NK1R-T signaling is of major importance in immune activation.[77,84] These observations support our overall hypothesis that, by expressing the truncated isoform in immune cells, NK1R alternative splicing is a negative regulator of SP signaling. The expression of NK1R-T prevents excessive activation of immune cells when exposed to low SP concentrations and down-regulates the SP signaling response in instances when SP levels are higher; which occurs under a variety of pathologic conditions.

NK1R-T is expressed in monocytes, macrophages, neutrophils, dendritic cells, eosinophils, T-cells, NK, and mast cells (for review see Ref. 33). In contrast to immune cells, NK1R-F transcripts were detected in platelets and its presence correlated with increased clot formation.[85] Caberlotto et al.[48] generated a real-time RT-PCR-based assay capable of distinguishing NK1R isoforms and provided data on NK1R isoform mRNA expression in different tissues. In most tissues, both isoforms were present although at different ratios, the authors were unable to detect NK1R-F transcripts in lymphocytes or in macrophages. Most studies detected total NK1R in lymphoid cells, but did not distinguish between full-length and truncated isoforms in lymphoid cells. In NK cells, the low levels of NK1R-F transcripts were detected.[67] Different concentrations of SP are required to elicit specific cellular functional response. For example, 10 nM of SP increased NK cell chemotaxis,[86] whereas 1–10 μM of SP was required to alter NK cell cytotoxicity.[67,86,87] In T-cells, 1–10 μM of SP was required in order to elicit T helper 17 cell polarization.[66]

In human lymphoid cells, the low expression levels of NK1R may also affect the ability to measure receptor activity. The expression level of NK1R in human lamina propria mononuclear cells is 7.5 ± 2.2 mRNA transcripts per cell. The number of NK1R transcripts is even lower in circulating PBMCs.[88] However, using samples from patients enrolled in our clinical study *Depression Antidepressants and HIV Infectivity*, we performed quantitative PCR, and we were able to detect NK1R-T but not NK1R-F mRNA transcripts in monocyte-derived macrophages (MDMs) from 58 out of 60 samples.[89] In human cell lines, non-stimulated monocytoid THP-1 cells express NK1R-T, whereas after PMA induced maturation, they express both NK1R-F and NK1R-T isoforms.[90] We were unable to demonstrate NK1R-F in primary human monocyte/macrophages treated under different conditions *in vitro* (data not shown). However, NK1R-F may be expressed *in vivo*. For example, in alveolar macrophage cultures from lung tissue obtained from tobacco smokers, a NK1R isoform corresponding to the full-length receptor, based on the molecular weight of the product, was detected.[91] Moreover, in alveolar macrophages, stimulation with nanomolar concentrations of SP resulted in IL-1β production and NFkB activation, suggesting that this effect is mediated by NK1R-F.[91]

SP interactions with the immune system generally result in pro-inflammatory responses.[4,40,92,93] The treatment of human macrophages with SP affects macrophage polarization. For example, exposure of human monocytes/MDMs to SP promotes a distinctive phenotype with features of both M1 and M2 polarization. Initially, within several hours of SP treatment, there is classical M1 polarization, which is linked to a pro-inflammatory response and increased production of cytokines, including IL-12 and TNFα. However, at 4–6 days after SP treatment, we observe an increase of CD163 and IL-10 expression, which is characteristic of M2 polarization.[65] This duality, may explain some of the discrepancies reported on the effect of SP on the overall immune response. The majority of publications claim a pro-inflammatory effect following SP exposure (for review see Refs. 4,40), several studies however, show protective properties of SP, possibly by driving macrophages toward the M2 phenotype through the up-regulation of IL-4 and IL-10.[35,37,38] In a recent publication, using rat bone marrow MDM cultures, SP skews macrophage polarization toward the M2 phenotype through activation of the PI3K/Akt/mTOR/S6 kinase pathway and induction of Arginase-1, CD163, and CD206. Relatedly, adoptively transferred SP polarized macrophages, also exhibited M2 polarization characteristics, by improving functional recovery in a rat spinal cord injury model.[94]

The SP-induced monocyte/MDM polarization has important functional ramifications. For instance, it creates favorable conditions for productive HIV infection in macrophages.[65] Elevated plasma levels of SP have been observed in the plasma of HIV infected individuals.[95,96] Overall, the effect of SP on the immune system is associated with increases in inflammatory responses and it mediates various outcomes on many immunologic conditions such as viral, bacterial, fungal and parasitic infections, and autoimmune diseases (for review see Refs. 4,33). NK1R antagonists are promising therapeutic candidates.

The NK1R antagonist, aprepitant, has been approved for human use, for chemotherapy-induced nausea and vomiting. NK1R antagonists have been tested in human clinical trials for depression, insomnia, and post-traumatic stress disorder with mixed or negative results (for review see Refs. 84,97,98). However, most studies targeted the NK1R-F isoform expressed in neurons. On the other hand, several studies showed that NK1R antagonists reduced cytokine production and decreased overall inflammation *in vivo*.[4,40,77,99] In 1 of 4 human clinical studies, we showed that treatment with aprepitant resulted in significant decreases in several pro-inflammatory markers including TNFα, MIP-1α, G-CSF, IL-6, IL-8, and sCD163 after 2 weeks of administration compared with the placebo group.[77] Spitsin et al.[99] recently demonstrated, using proteomics, that aprepitant modulated inflammatory pathways in aviremic HIV infected individuals. Thus we have conclusively demonstrated that NK1R antagonism *in vivo* results in down-regulation of inflammation in HIV infected individuals.[77,99,100]

6 | NK1R ALTERNATIVE SPLICING IN CANCER

In the immune system, NK1R alternative splicing is a negative regulator of SP signaling. NK1R-T abrogates immune cell activation and modulates immune function. In cancer, the pathophysiologic importance of SP/NK1R signaling has become evident. In tumor cells, SP promotes cell proliferation, migration, survival, and metastasis.[33] NK1R signaling is associated with plasma membrane blebbing,[101] which may also contribute to cancer metastasis.[102]

Several types of cancer cells overexpress NK1R and, in many cases, tumor cells overexpress NK1R receptors in comparison to normal, non-tumor tissue (for review see Ref. 33). The inability of NK1R-T to be regulated through internalization or desensitization (Fig. 2) may be the basis for the enhanced plasma membrane expression of NK1R in tumor cells. In several types of cancer, the expression of NK1R-T but not NK1R-F is associated with increased malignant transformation.[8–11] However, NK1R-T plays a different role in cancer compared with the immune system. This role may be attributed to several factors, including differences in NK1R expression levels (while many cancer cells overexpress NK1R, immune cells maintain very low NK1R levels), unknown differences in downstream signaling, or reduced NK1R-T translocation to the nucleus which has been described for several types of cancer cells but not for immune cells[98].

NK1R antagonists reverse the effects of SP signaling. This effect offers an opportunity to target NK1R/SP interactions for novel anti-tumor therapies. NK1R antagonists are being investigated for their potential to restrict tumor cell growth and metastasis of several types of cancers (for review see Ref. 33). Data on the role of SP/NK1R signaling in cancer pathogenesis and disease progression are generated using *in vitro* assays and through functional studies in animal models. This approach may lead to clinical trials. As mentioned above, the NK1R antagonist, aprepitant, is approved by the FDA for human use as an antiemetic administered as a triple dose of 125, 80, and 80 mg during 3 consecutive days, though higher doses and longer administration may be required as an anticancer drug.

7 | CONCLUDING REMARKS

The 2 NK1R isoforms, full length and truncated, have distinct tissue expression patterns and differ in signal transduction activities. The truncated splice variant of NK1R requires 10–100-fold higher concentrations of its cognate ligand (SP) than NK1R-F for maximum activation. There are altered effects of SP signaling in cells which predominantly express NK1R-T including immune cells.

In tissues that express NK1R-F, such as the CNS, a receptor responsive to low ligand concentrations and capable of desensitization may be optimal for cell functions. In the immune system, a highly efficient receptor may produce potentially harmful inflammation and immune activation. Immune cells express predominantly NK1R-T, resulting in diminished SP/NK1R signaling and thus more finely tuned immune signaling. The biologic consequences are operative on several immunomodulatory pathways that are affected by NK1R signaling, such as macrophage/monocyte polarization and NK cell signaling.

The effects of NK1R alternative splicing can be particularly applicable in the pathophysiologic mechanism of inflammatory, infectious, and autoimmune diseases. These conditions are associated with increased SP levels and therefore could engage in SP/NK1R-T signaling. In other diseases, such as cancer, the altered properties of the NK1R-T isoform may be involved in disease pathogenesis in a completely different way. In such cases, the altered signaling of NK1R-T may be favorable for cancer cell proliferation and this property is potentially being exploited by the disease mechanism through overexpression of the truncated isoform.

In summary, alternative splicing of NK1R is important to maintain immune system function. In addition, disturbances of the SP/NK1R-T signaling pathway may be implicated in a variety of pathophysiologic conditions. The different properties of NK1R isoforms should be further studied and taken into account when considering therapeutic approaches that target NK1R signaling.

ACKNOWLEDGMENTS

We thank Sue Seif, MA, CMI medical illustrator for her distinguished graphics and Dr. Zenobia Cofer, PhD, for her excellent editorial assistance.

We thank the faculty, staff, and students of the Dr. Steven D. Douglas, MD, laboratory for their experiments, insights, and dedication to the investigation of NK1R and SP interactions. We applaud Dr. Susan E. Leeman, PhD, for her seminal contributions to tachykinin and NK1R biology. We are grateful to Dr. Leeman for her extraordinary insights and dedicate this mini review to Dr. S.E. Leeman for her major contributions to the life sciences.

The authors S.S., V.P., and S.D.D. were supported by the grants from National Institutes of Health (RO1 AI106509, UO1 MH090325, P30 MH097488, and PO1 MH105303.

DISCLOSURES

The authors declare no conflicts of interest.

REFERENCES

1. Yokota Y, Sasai Y, Tanaka K, et al. Molecular characterization of a functional cDNA for rat substance P receptor. *J Biol Chem*. 1989;264:17649–17652.
2. Maggi CA. The mammalian tachykinin receptors. *Gen Pharmacol*. 1995;26:911–944.
3. Pennefather JN, Lecci A, Candenas ML, Patak E, Pinto FM, Maggi CA. Tachykinins and tachykinin receptors: A growing family. *Life Sci*. 2004;74:1445–1463.
4. Douglas SD, Leeman SE. Neurokinin-1 receptor: Functional significance in the immune system in reference to selected infections and inflammation. *Ann N Y Acad Sci*. 2011;1217:83–95.
5. Douglas SD, Leeman SE, Heyward CY, et al, Tachykinin receptors. IUPHAR/BPS Guide to PHARMACOLOGY. Retrieved from http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId = 62
6. Kilpatrick GJ, Dautzenberg FM, Martin GR, Eglen RM. 7TM receptors: The splicing on the cake. *Trends Pharmacol Sci*. 1999;20:294–301.
7. Markovic D, Challiss RA. Alternative splicing of G protein-coupled receptors: Physiology and pathophysiology. *Cell Mol Life Sci*. 2009;66:3337–3352.
8. Gillespie E, Leeman SE, Watts LA, et al. Truncated neurokinin-1 receptor is increased in colonic epithelial cells from patients with colitis-associated cancer. *Proc Natl Acad Sci USA*. 2011;108:17420–17425.
9. Zhou Y, Zhao L, Xiong T, et al. Roles of full-length and truncated neurokinin-1 receptors on tumor progression and distant metastasis in human breast cancer. *Breast Cancer Res Treat*. 2013;140:49–61.
10. Garnier A, Ilmer M, Becker K, Haberle BDVONS, Kappler R, Berger M. Truncated neurokinin-1 receptor is an ubiquitous antitumor target in hepatoblastoma, and its expression is independent of tumor biology and stage. *Oncol Lett*. 2016;11:870–878.
11. Patel HJ, Ramkissoon SH, Patel PS, Rameshwar P. Transformation of breast cells by truncated neurokinin-1 receptor is secondary to activation by preprotachykinin-A peptides. *Proc Natl Acad Sci USA*. 2005;102:17436–17441.
12. Pohl A, Kappler R, Muhling J, VON Schweinitz D, Berger M. Expression of truncated neurokinin-1 receptor in childhood neuroblastoma is independent of tumor biology and stage. *Anticancer Res*. 2017;37:6079–6085.
13. Rossi L, Iacopetti P, Salvetti A. Stem cells and neural signalling: The case of neoblast recruitment and plasticity in low dose X-ray treated planarians. *Int J Dev Biol*. 2012;56:135–142.
14. Li XJ, Wolfgang W, Wu YN, North RA, Forte M. Cloning, heterologous expression and developmental regulation of a Drosophila receptor for tachykinin-like peptides. *EMBO J*. 1991;10:3221–3229.
15. Ui-Tei K, Sakuma M, Watanabe Y, Miyake T, Miyata Y. Chemical analysis of neurotransmitter candidates in clonal cell lines from Drosophila central nervous system, II: Neuropeptides and amino acids. *Neurosci Lett*. 1995;195:187–190.
16. Hu G, He M, Ko WKW, Wong AOL. TAC1 gene products regulate pituitary hormone secretion and gene expression in prepubertal grass carp pituitary cells. *Endocrinology*. 2017;158:1776–1797.
17. vonEuler, U. S. and Gaddum, J. H. An unidentified depressor substance in certain tissue extracts. *J Physiol* 1931;72:74–87.
18. Chang MM, Leeman SE. Isolation of a sialogogic peptide from bovine hypothalamic tissue and its characterization as substance P. *J Biol. Chem*. 1970;245:4784–4790.
19. Chang MM, Leeman SE, Niall HD. Amino-acid sequence of substance P. *Nat New Biol*. 1971;232:86–87.
20. Bost KL. Tachykinin-mediated modulation of the immune response. *Front Biosci*. 2004;9:3331–3332.
21. Vink R, Gabrielian L, Thornton E. The role of substance P in secondary pathophysiology after traumatic brain injury. *Front Neurol*. 2017;8:304.
22. Ho WZ, Lai JP, Zhu XH, Uvaydova M, Douglas SD. Human monocytes and macrophages express substance P and neurokinin-1 receptor. *J Immunol*. 1997;159:5654–5660.
23. Satake H, Kawada T. Overview of the primary structure, tissue-distribution, and functions of tachykinins and their receptors. *Curr Drug Targets*. 2006;7:963–974.
24. Leeman SE, Ferguson SL. Substance P: An historical perspective. *Neuropeptides*. 2000;34:249–254.
25. Bremer AA, Leeman SE. Substance P. *Encyclopedia of Life Sciences (ELS)*. Chichester: John Wiley & Sons, Ltd; 2010.
26. Goodman A. Neurobiology of addiction. An integrative review. *Biochem Pharmacol*. 2008;75:266–322.
27. Suvas S. Role of substance P neuropeptide in inflammation, wound healing, and tissue homeostasis. *J Immunol*. 2017;199:1543–1552.
28. Nelson DA, Marriott I, Bost KL. Expression of hemokinin 1 mRNA by murine dendritic cells. *J Neuroimmunol*. 2004;155:94–102.
29. O'Connor TM, O'Connell J, O'Brien DI, Goode T, Bredin CP, Shanahan F. The role of substance P in inflammatory disease. *J Cell Physiol*. 2004;201:167–180.
30. Weinstock JV, Blum A, Metwali A, Elliott D, Bunnett N, Arsenescu R. Substance P regulates Th1-type colitis in IL-10 knockout mice. *J Immunol*. 2003;171:3762–3767.
31. Guo CJ, Lai JP, Luo HM, Douglas SD, Ho WZ. Substance P upregulates macrophage inflammatory protein-1beta expression in human T lymphocytes. *J Neuroimmunol*. 2002;131:160–167.
32. Steinhoff MS, von Mentzer B, Geppetti P, Pothoulakis C, Bunnett NW. Tachykinins and their receptors: Contributions to physiological control and the mechanisms of disease. *Physiol Rev*. 2014;94:265–301.
33. Munoz M, Covenas R. Involvement of substance P and the NK-1 receptor in human pathology. *Amino Acids*. 2014;46:1727–1750.
34. Mashaghi A, Marmalidou A, Tehrani M, Grace PM, Pothoulakis C, Dana R. Neuropeptide substance P and the immune response. *Cell Mol Life Sci*. 2016;73:4249–4264.
35. Hong HS, Hwang DY, Park JH, Kim S, Seo EJ, Son Y. Substance-P alleviates dextran sulfate sodium-induced intestinal damage by suppressing inflammation through enrichment of M2 macrophages and regulatory T cells. *Cytokine*. 2017;90:21–30.
36. Montana G, Lampiasi N. Substance P induces HO-1 expression in RAW 264.7 cells promoting switch towards M2-like macrophages. *PloS One*. 2016;11:e0167420.
37. Jiang MH, Lim JE, Chi GF, et al. Substance P reduces apoptotic cell death possibly by modulating the immune response at the early stage after spinal cord injury. *Neuroreport*. 2013;24:846–851.
38. Jiang MH, Chung E, Chi GF, et al. Substance P induces M2-type macrophages after spinal cord injury. *Neuroreport*. 2012;23:786–792.
39. Vinet-Oliphant H, Alvarez X, Buza E, et al. Neurokinin-1 receptor (NK1-R) expression in the brains of SIV-infected rhesus macaques: Implications for substance P in NK1-R immune cell trafficking into the CNS. *Am J Pathol*. 2010;177:1286–1297.
40. Tuluc F, Lai JP, Kilpatrick LE, Evans DL, Douglas SD. Neurokinin 1 receptor isoforms and the control of innate immunity. *Trends Immunol*. 2009;30:271–276.

41. Fong TM, Anderson SA, Yu H, Huang RR, Strader CD. Differential activation of intracellular effector by two isoforms of human neurokinin-1 receptor. *Mol Pharmacol*. 1992;41:24–30.
42. Boyd ND, Kage RK, Leeman SE. Characterization of the NK1 receptor using photoaffinity probes. In: Buck S H, ed. *The Tachykinin Receptors*. Humana Press; 1994:219–236.
43. Baker SJ, Morris JL, Gibbins IL. Cloning of a C-terminally truncated NK-1 receptor from guinea-pig nervous system. *Brain Res Mol Brain Res*. 2003;111:136–147.
44. Liu L, Markus I, Vandenberg RJ, Neilan BA, Murray M, Burcher E. Molecular identification and characterization of three isoforms of tachykinin NK(1)-like receptors in the cane toad Bufo marinus. *Am J Physiol Regul Integr Compar Physiol*. 2004;287:R575–585.
45. Randolph GP, Simon JS, Arreaza MG, Qiu P, Lachowicz JE, Duffy RA. Identification of single-nucleotide polymorphisms of the human neurokinin 1 receptor gene and pharmacological characterization of a Y192H variant. *Pharmacogenomics J*. 2004;4:394–402.
46. Wang Y, Liu J, Huang BO, et al. Mechanism of alternative splicing and its regulation. *Biomed Rep*. 2015;3:152–158.
47. Oladosu FA, Maixner W, Nackley AG. Alternative splicing of G protein-coupled receptors: Relevance to pain management. *Mayo Clin Proc*. 2015;90:1135–1151.
48. Caberlotto L, Hurd YL, Murdock P, et al. Neurokinin 1 receptor and relative abundance of the short and long isoforms in the human brain. *Eur J Neurosci*. 2003;17:1736–1746.
49. Mantyh PW, Rogers SD, Ghilardi JR, Maggio JE, Mantyh CR, Vigna SR. Differential expression of two isoforms of the neurokinin-1 (substance P) receptor in vivo. *Brain Res*. 1996;719:8–13.
50. Flor PJ, Gomeza J, Tones MA, Kuhn R, Pin JP, Knopfel T. The C-terminal domain of the mGluR1 metabotropic glutamate receptor affects sensitivity to agonists. *J Neurochem*. 1996;67:58–63.
51. Hermans E, Challiss RA. Structural, signalling and regulatory properties of the group I metabotropic glutamate receptors: Prototypic family C G-protein-coupled receptors. *Biochem J*. 2001;359:465–484.
52. Jin J, Mao GF, Ashby B. Constitutive activity of human prostaglandin E receptor EP3 isoforms. *Br J Pharmacol*. 1997;121:317–323.
53. Bohm SK, Khitin LM, Smeekens SP, Grady EF, Payan DG, Bunnett NW. Identification of potential tyrosine-containing endocytic motifs in the carboxyl-tail and seventh transmembrane domain of the neurokinin 1 receptor. *J Biol Chem*. 1997;272:2363–2372.
54. Li H, Leeman SE, Slack BE, et al. A substance P (neurokinin-1) receptor mutant carboxyl-terminally truncated to resemble a naturally occurring receptor isoform displays enhanced responsiveness and resistance to desensitization. *Proc Natl Acad Sci USA*. 1997;94:9475–9480.
55. Akazawa T, Kwatra SG, Goldsmith LE, et al. A constitutively active form of neurokinin 1 receptor and neurokinin 1 receptor-mediated apoptosis in glioblastomas. *J Neurochem*. 2009;109:1079–1086.
56. Schmidlin F, Roosterman D, Bunnett NW. The third intracellular loop and carboxyl tail of neurokinin 1 and 3 receptors determine interactions with beta-arrestins. American journal of physiology. *Cell Physiol*. 2003;285:C945–958.
57. Holst B, Hastrup H, Raffetseder U, Martini L, Schwartz TW. Two active molecular phenotypes of the tachykinin NK1 receptor revealed by G-protein fusions and mutagenesis. *J Biol Chem*. 2001;276:19793–19799.
58. Almeida TA, Rojo J, Nieto PM, et al. Tachykinins and tachykinin receptors: Structure and activity relationships. *Curr Med Chem*. 2004;11:2045–2081.
59. Fong TM, Huang RC, Yu H, et al. Mutational analysis of neurokinin receptor function. *Can J Physiol Pharmacol*. 1995;73:860–865.
60. Sanders MA. Desensitization of the neurokinin 1 receptor is mediated by the receptor carboxy-terminal region, but is not caused by receptor internalization. *J Neurochem*. 1996;67:2362–2372.
61. Vigna SR. Phosphorylation and desensitization of neurokinin-1 receptor expressed in epithelial cells. *J Neurochem*. 1999;73:1925–1932.
62. Dery O, Defea KA, Bunnett NW. Protein kinase C-mediated desensitization of the neurokinin 1 receptor. *Am J Physiol Cell Physiol*. 2001;280:C1097–106.
63. Sagan S, Karoyan P, Chassaing G, Lavielle S. Further delineation of the two binding sites (R*(n)) associated with tachykinin neurokinin-1 receptors using [3-Prolinomethionine(11)]SP analogues. *J Biol Chem*. 1999;274:23770–23776.
64. Spitsin S, Tuluc F, Meshki J, Ping Lai J, Tustin Iii R, Douglas SD. Analog of somatostatin vapreotide exhibits biological effects in vitro via interaction with neurokinin-1 receptor. *Neuroimmunomodulation*. 2013;20:247–255.
65. Tuluc F, Meshki J, Spitsin S, Douglas SD. HIV infection of macrophages is enhanced in the presence of increased expression of CD163 induced by substance P. *J Leukoc Biol*. 2014;96:143–150.
66. Cunin P, Caillon A, Corvaisier M, et al. The tachykinins substance P and hemokinin-1 favor the generation of human memory Th17 cells by inducing IL-1beta, IL-23, and TNF-like 1A expression by monocytes. *J Immunol*. 2011;186:4175–4182.
67. Monaco-Shawver L, Schwartz L, Tuluc F, et al. Substance P inhibits natural killer cell cytotoxicity through the neurokinin-1 receptor. *J Leukoc Biol*. 2011;89:113–125.
68. Tuluc F, Meshki J, Spitsin S, Douglas SD. HIV infection of macrophages is enhanced in the presence of increased expression of CD163 induced by substance P. *J Leukoc Biol*. 2014.
69. Spitsin S, Meshki J, Winters A, Tuluc F, Benton TD, Douglas SD. Substance P-mediated chemokine production promotes monocyte migration. *J Leukoc Biol*. 2017;101:967–973.
70. Cailes J, Winter S, du Bois RM, Evans TW. Defective endothelially mediated pulmonary vasodilation in systemic sclerosis. *Chest*. 1998;114:178–184.
71. Conlon JM, Sheehan L. Conversion of substance P to C-terminal fragments in human plasma. *Regul Pept*. 1983;7:335–345.
72. Duggan AW. Release of neuropeptides in the spinal cord. *Prog Brain Res*. 1995;104:197–223.
73. Seelig A. Substance P and antagonists. Surface activity and molecular shapes. *Biochim Biophys Acta*. 1990;1030:111–118.
74. Seelig A, Doelz R. Substance P and antagonists. Surface activity, conformations, and lipid binding. *Ann N Y Acad Sci*. 1991;632:468–470.
75. Vilisaar J, Kawabe K, Braitch M, et al. Reciprocal regulation of substance P and IL-12/IL-23 and the associated cytokines, IFNgamma/IL-17: A perspective on the relevance of this interaction to multiple sclerosis. *J Neuroimmune Pharmacol*. 2015;10:457–467.
76. Lai JP, Lai S, Tuluc F, et al. Differences in the length of the carboxyl terminus mediate functional properties of neurokinin-1 receptor. *Proc Natl Acad Sci USA*. 2008;105:12605–12610.
77. Tebas P, Spitsin S, Barrett JS, et al. Reduction of soluble CD163, substance P, programmed death 1 and inflammatory markers: Phase 1B trial of aprepitant in HIV-1-infected adults. *Aids*. 2015;29:931–939.
78. Chernova I, Lai JP, Li H, et al. Substance P (SP) enhances CCL5-induced chemotaxis and intracellular signaling in human monocytes, which express the truncated neurokinin-1 receptor (NK1R). *J Leukoc Biol*. 2009;85:154–164.
79. Jakab RL, Hazrati LN, Goldman-Rakic P. Distribution and neurochemical character of substance P receptor (SPR)-immunoreactive

striatal neurons of the macaque monkey: Accumulation of SP fibers and SPR neurons and dendrites in "striocapsules" encircling striosomes. *J Compar Neurol*. 1996;369:137–149.

80. Li JL, Wang D, Kaneko T, Shigemoto R, Nomura S, Mizuno N. Relationship between neurokinin-1 receptor and substance P in the striatum: Light and electron microscopic immunohistochemical study in the rat. *J Compar Neurol*. 2000;418:156–163.
81. Lai JP, Cnaan A, Zhao H, Douglas SD. Detection of full-length and truncated neurokinin-1 receptor mRNA expression in human brain regions. *J Neurosci Methods*. 2008;168:127–133.
82. Douglas SD, Lynch KG, Lai JP. Neurokinin-1 receptor mRNA expression differences in brains of HIV-infected individuals. *J Neurol Sci*. 2008;272:174–177.
83. Spitsin S, Stevens KE, Douglas SD. Expression of substance P, neurokinin-1 receptor and immune markers in the brains of individuals with HIV-associated neuropathology. *J Neurol Sci*. 2013;334:18–23.
84. Barrett JS, Spitsin S, Moorthy G, et al. Pharmacologic rationale for the NK1R antagonist, aprepitant as adjunctive therapy in HIV. *J Translat Med*. 2016;14:148.
85. Azma T, Sugimoto Y, Kinoshita H, et al. Detection of the full-length transcript variant for neurokinin-1 receptor in human whole blood associated with enhanced reinforcement of clot by substance-P. *J Thromb Thromb*. 2012;33:329–337.
86. Feistritzer C, Clausen J, Sturn DH, et al. Natural killer cell functions mediated by the neuropeptide substance P. *Regul Pept*. 2003;116:119–126.
87. Flageole H, Senterman M, Trudel JL. Substance P increases in vitro lymphokine-activated-killer (LAK) cell cytotoxicity against fresh colorectal cancer cells. *J Surg Res*. 1992;53:445–449.
88. Goode T, O'Connell J, Ho WZ, et al. Differential expression of neurokinin-1 receptor by human mucosal and peripheral lymphoid cells. *Clin Diagn Lab Immunol*. 2000;7:371–376.
89. Greeson JM, Gettes DR, Spitsin S, et al. The selective serotonin reuptake inhibitor citalopram decreases human immunodeficiency virus receptor and coreceptor expression in immune cells. *Biol Psychiatry*. 2016;80:33–39.
90. Lai JP, Ho WZ, Kilpatrick LE, et al. Full-length and truncated neurokinin-1 receptor expression and function during monocyte/macrophage differentiation. *Proc Natl Acad Sci USA*. 2006;103:7771–7776.
91. Bardelli C, Gunella G, Varsaldi F, et al. Expression of functional NK1 receptors in human alveolar macrophages: Superoxide anion production, cytokine release and involvement of NF-kappaB pathway. *Br J Pharmacol*. 2005;145:385–396.
92. Mashaghi A, Marmalidou A, Tehrani M, Grace PM, Pothoulakis C, Dana R. Neuropeptide substance P and the immune response. *Cell Mol Life Sci*. 2016.
93. Cattaruzza F, Poole DP, Bunnett NW. Arresting inflammation: Contributions of plasma membrane and endosomal signalling to neuropeptide-driven inflammatory disease. *Biochem Soc Trans*. 2013;41:137–143.
94. Lim JE, Chung E, Son Y. A neuropeptide, Substance-P, directly induces tissue-repairing M2 like macrophages by activating the PI3K/Akt/mTOR pathway even in the presence of IFNgamma. *Sci Rep*. 2017;7:9417.
95. Douglas SD, Ho WZ, Gettes DR, et al. Elevated substance P levels in HIV-infected men. *Aids*. 2001;15:2043–2045.
96. Douglas SD, Cnaan A, Lynch KG, et al. Elevated substance P levels in HIV-infected women in comparison to HIV-negative women. *AIDS Res Hum Retroviruses*. 2008;24:375–378.
97. Rupniak NMJ, Kramer MS. NK1 receptor antagonists for depression: Why a validated concept was abandoned. *J Affect Disord*. 2017;223:121–125.
98. Quartara L, Altamura M, Evangelista S, Maggi CA. Tachykinin receptor antagonists in clinical trials. *Exp Opin Invest Drugs*. 2009;18:1843–1864.
99. Spitsin S, Tebas P, Barrett JS, et al. Antiinflammatory effects of aprepitant coadministration with cART regimen containing ritonavir in HIV-infected adults. *JCI Insight*. 2017;2.
100. Tebas P, Tuluc F, Barrett JS, et al. A randomized, placebo controlled, double masked phase IB study evaluating the safety and antiviral activity of aprepitant, a neurokinin-1 receptor antagonist in HIV-1 infected adults. *PloS One*. 2011;6:e24180.
101. Chen P, Douglas SD, Meshki J, Tuluc F. Neurokinin 1 receptor mediates membrane blebbing and sheer stress-induced microparticle formation in HEK293 cells. *PloS One*. 2012;7:e45322.
102. Paluch EK, Raz E. The role and regulation of blebs in cell migration. *Curr Opin Cell Biol*. 2013;25:582–590.

How to cite this article: Spitsin S, Pappa V, Douglas SD. Truncation of neurokinin-1 receptor—negative regulation of substance P signaling. *J Leukoc Biol*. 2018;103:1043–1051. https://doi.org/10.1002/JLB.3MIR0817-348R