



Neuropeptide substance P and the immune response

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Abstract Substance P is a peptide mainly secreted by neurons and is involved in many biological processes, including nociception and inflammation. Animal models have provided insights into the biology of this peptide and offered compelling evidence for the importance of substance P in cell-to-cell communication by either paracrine or endocrine signaling. Substance P mediates interactions between neurons and immune cells, with nerve-derived substance P modulating immune cell proliferation rates and cytokine production. Intriguingly, some immune cells have also been found to secrete substance P, which hints at an integral role of substance P in the immune response. These communications play important functional roles in immunity including mobilization, proliferation and modulation of the activity of immune cells. This review summarizes current knowledge of substance P and its receptors, as well as its physiological and pathological roles. We focus on recent developments in the immunobiology of substance P and discuss the clinical implications of its ability to modulate the immune response.

Keywords Immune regulation · Neuropeptides · Cell-to-cell communication · Signaling · Cellular dynamics

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Introduction

Substance P (SP) is a highly conserved peptide that was originally discovered in 1931 by Von Euler and Gaddum in the equine brain and gut extracts—distinct from acetylcholine—capable of inducing hypotension and muscle contraction [1]. This substance was purified and dried in powder form (hence the name substance P) [2]; highly conserved homologs were later identified in mice, rabbits, and humans (Fig. 1a). SP is encoded by the *TAC1* gene (located on chromosome 7 in humans) and is a member of the tachykinin peptide hormone family [3] (Fig. 1b); the family also contains three other neuropeptides, also encoded by *TAC1*, namely neurokinin A, neuropeptide K, and neuropeptide γ [4, 5]. SP is expressed by many cell types including neurons [6–9], astrocytes [10, 11], microglia [12], epithelial cells [13], and endothelial cells [14]. Immune cells, such as T cells [15], macrophages [16, 17], dendritic cells [18], or eosinophils [19] also display significant levels of SP expression [16, 15, 20]. SP is also expressed by some stem cells and progenitor cells [21], including immunomodulatory mesenchymal stem cells (MSC) [22]. Such widespread expression of SP in diverse cell types may suggest its participation in a wide variety of physiological and pathophysiological functions, by activating a multitude of signaling pathways.

Structure

The physicochemical properties of substance P underlie its function. The SP peptide comprises 11 amino acids (RPKPQFFGLM-NH₂) [23] with a net positive charge at physiologic pH. Positively charged residues are located on the N-terminus, while the C-terminus contains hydrophobic

residues; this separation renders SP as an amphiphilic peptide (Fig. 1b). The amphiphilic nature of substance P governs its direct interaction with lipid bilayer membranes, but the functional importance of this interaction is not clear. Importantly, SP mediates its functions by interacting specifically with surface receptors, i.e. members of the neurokinin (NK) family of G-protein coupled receptors (Fig. 1c).

Substance P is stable in plasma, but has a short half-life in tissues. The half-life of SP is defined by the kinetics of chemical or enzymatic degradation in the extracellular environment, by binding to cells, and by the dynamics of cellular internalization. Reported values for SP half-life are in the range of seconds to tens of minutes in tissues and blood, while in extracted blood plasma SP is stable on the time scale of hours [24–26].

Neurokinin receptors

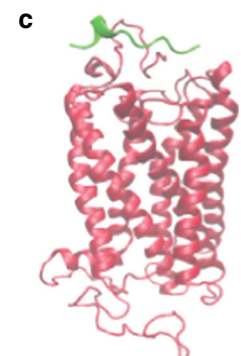
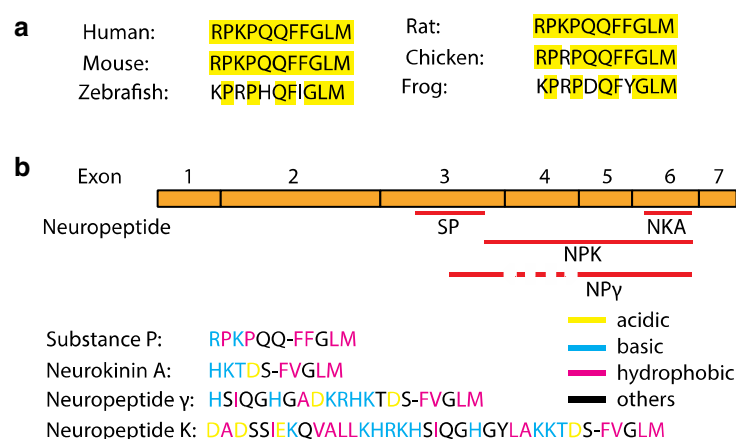
NK receptors are of three canonical types, namely NK1R, NK2R, and NK3R, often co-expressed by the same cell. Among these, NK1R, which typically localizes to lipid rafts [27], is a G-protein coupled receptor and displays the highest affinity for substance P. This receptor has two isoforms with differing affinities: a high-affinity receptor, NK1R-F (407 residue-long full-length version) and the low-affinity NK1R-T (311 residue-long truncated version) [28–30]. The full-length NK1R (NK1R-F) is the predominant form expressed at certain sites in the human brain, whereas the truncated NK1R (NK1R-T) is widespread throughout the central nervous system and in peripheral tissues [31]. The activation of NK1R by SP depends on the microarchitecture and the composition of the plasma membrane. For example, when cholesterol is depleted, the NK1R-mediated signaling is abolished [27]. The NK1R receptor isoforms are expressed by neurons [32, 33], epithelial [34], endothelial [35], and smooth muscle cells [36], as well as fibroblasts [37]. Intriguingly, some immune

cells including T and B lymphocytes [15], natural killer cells [38], dendritic cells [39], monocytes/macrophages [40, 16], microglia and astrocytes [41], eosinophils and mast cells express NK1R [42]. It remains unclear why cells express three different classes of NK receptor family with varying affinities, and what benefit this complexity confers to cells.

Regulatory mechanisms

Interaction of substance P with its receptors leads to internalization and recycling of the receptor, a process that regulates the sensitivity of the cell to SP. Desensitization is mediated by two proteins, a kinase and β -arrestin, and is followed by rapid internalization [43]. Resensitization involves pH-induced dephosphorylation and recycling of NK1R (Fig. 2, right panel) [44]. Activation of NK1R stimulates translocation of G-protein coupled-receptor kinases (GRKs) from the cytosol to the plasma membrane where they specifically phosphorylate SP-occupied NK1R molecules (Fig. 2, right panel) [45]. β -Arrestins also translocate to the plasma membrane, where they interact with the phosphorylated NK1R [46]. The SP/NK1R- β -arrestin complex is rapidly internalized and subsequently exposed to an acidic environment, which hydrolyzes phosphate groups from NK1R. Substance P is then detached from NK1R and degraded by proteolytic enzymes; in turn, NK1R recycles to the cell surface, leading to resensitization [44]. The process of de/re-sensitization typically (but not always) involves internalization and recycling and is tightly regulated. The phosphorylation of NK1R by GRKs depends on the concentration of available SP. NK1R undergoes extensive phosphorylation and prolonged association with β -arrestins for hours, following exposure to high concentrations of SP (>10 nM). In contrast, minimal phosphorylation is seen after exposure to low concentrations of SP (<1 nM) [47, 48]. Transforming growth factor β (TGF- β) delays SP-induced NK1R

Fig. 1 Molecular properties of substance P. **a** The primary sequence of substance P is conserved across species. **b** TAC1 gene encodes substance P along with three other neuropeptides with significant similarities in primary sequences. *One-letter notation* is used for amino acids. **c** Structure of NK1R bound to substance P (PDB = 2KS9)



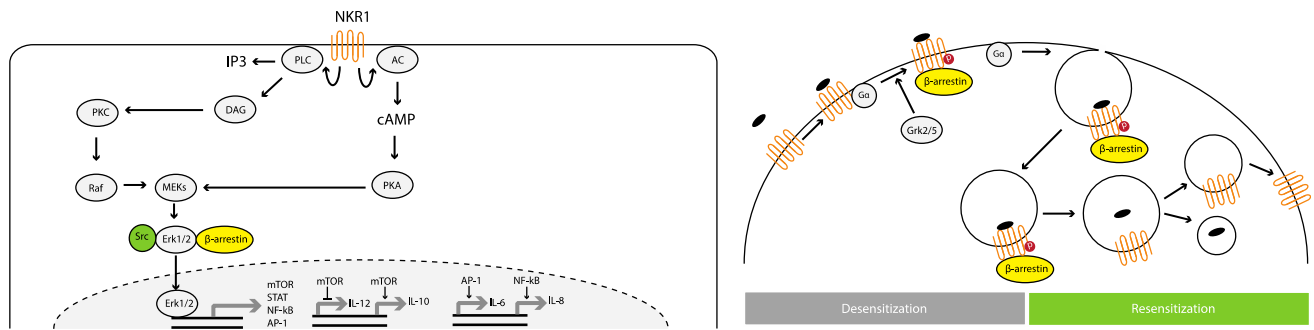


Fig. 2 NK1R signaling and regulation. *Left* NK1R-associated signaling pathways control the expression of cytokines and transcription factors with critical involvement in immune regulation. *Right* high concentration of SP (black oval) initiates a desensitization process

internalization and thus enhances the activity of NK1R in T cells [49], whereas inflammatory cytokines typically promote upregulation of NK1R [50, 51]. Resensitization is regulated at a number of levels. An intracellular enzyme, endothelin-converting enzyme-1, controls the translocation of the receptors back to the surface; the enzyme is in turn subject to post-translational regulation [52, 53]. Recycling of NK1R depends on its ubiquitination state, which determines whether the internalized receptor is degraded or returns to the cellular membrane. Further, re-sensitization critically depends on Ras-related proteins (RABs), including RAB4a, RAB5a and RAB11a [47]. RABs are themselves subject to a number of regulatory mechanisms [54, 55]. Previously, we mentioned that NK1R-F shows higher affinity to SP than NK1R-T, potentially due to the role of the C-terminal domain in NK1R folding [56]. Interestingly, the relatively lower affinity of the NK1R-T to SP might impair its desensitization and internalization leading to more persistent responses after receptor–ligand interactions (reviewed in [56]). Finally, while in the majority of cases NK1 receptors are recycled via the mechanisms explained above, for a subpopulation of NK1 receptors, desensitization and resensitization may take place without receptor internalization and surface return, respectively [57].

Signaling pathways

Interaction of substance P with NK1 receptors signals to several intracellular pathways (Fig. 2a). These pathways involve second messengers, such as diacyl-glycerol (DAG), inositol trisphosphate (IP_3), and cyclic adenosine monophosphate (cAMP), which control the expression of cytokines and modulate ion channel activities. SP–NK1R coupling activates phospholipase C and adenylate cyclase to generate DAG/ IP_3 and cAMP, respectively, and eventually signals to mitogen-activated protein kinases (known as MAPKK or MEKs). MEKs activate extracellular signal-

involving phosphorylation of NK1R and its prolonged binding to β-arrestin. The phosphorylated NK1R can be recycled via endocytosis and acidification within endosomes

related kinases 1/2 (ERK1/2), which translocate into the nucleus and mediate the expression of cytokines through the serine/threonine protein kinase, mammalian target of rapamycin (mTOR), as well as the transcription factors, such as AP-1 and NF-κB. Figure 2a highlights the pathways that regulate the expression of several cytokines, including the balance of anti-inflammatory interleukin (IL)-10 and proinflammatory IL-12. Importantly, NK1R regulates chemokines like CCL2, CCL4, CXCL2, and IL-8 via NF-κB, thereby recruiting immune cells to sites of inflammation [58–68].

Pathophysiology

SP exerts a wide range of physiological as well as pathological effects. The most widely known roles of SP are in nociception and neurogenic inflammation [69–72], both primarily mediated by the NK1R receptor. However, the diverse expression of NK1R on various non-neuronal cell types [16, 15] suggests other functions in addition to its role in pain, including growth-promoting effects on smooth muscle cells [73, 74], skin fibroblasts [73], and synovocytes [75]; regulating the integrity of the extracellular matrix by controlling the expression of matrix metalloproteinases and tissue inhibitors of metalloproteinase in fibroblasts [76]; regulating angiogenesis and vasodilation by controlling the release of nitric oxide [77–79]; and regulating bone metabolism [80]. SP is known to be involved in bone marrow fibrosis [25], tumor cell proliferation [81, 82], and inflammatory processes [83]. Neurogenic inflammation plays a major role in rheumatoid arthritis (RA). SP is significantly increased in synovial fluid in RA patients [84]. Elevated levels of SP and upregulated NK1R expression are seen in the rectum and colon of patients with inflammatory bowel disease (IBD) and correlate well with disease activity [83]. SP is a part of an immune-regulatory mechanism that amplifies inflammation at intestinal mucosal surfaces in the acute phase of IBD

[85]. In particular, the ability of human mesenteric pre-adipocytes from IBD patients to release IL-17A increases in response to SP. In psoriasis, a disease that is characterized by hyperproliferation of keratinocyte, elevated amounts of SP are seen in the skin. Importantly, SP stimulates mast cells to generate IL-1 family cytokines, which in turn stimulate keratinocyte proliferation [86]. Finally, current models suggest that SP generated by immune cells stimulates Th1 and Th17 autoreactive cells that migrate to the CNS where they target the nerves [87]. These and other evidences together indicate that SP is a significant player in autoimmunity.

Finally, the expression of SP receptors is markedly increased during infection, especially on lymphocytes and macrophages. In human immunodeficiency virus (HIV) infection, and activation of NK1R by SP contributes to increased HIV-1 infection in macrophages [56]. In the following sections, we elaborate on the immunobiology of SP and provide insights into the immunomodulatory roles of SP in disease processes.

Immunobiology of substance P

The immune system provides protection from a wide range of pathogens. One component of immunity, the phylogenetically ancient innate immune response, fights infections from the moment of first contact and is the fundamental and generic defensive weapon of both uni- and multicellular organisms [88]. An essential element of an immune response is the communication between cells. For example, after injury or infection, tissue resident cells such as nerves, vascular cells and immune cells actively interact in a paracrine or contact-dependent manner. These communications precede recruitment of non-resident immune cells and may lead to the development of adaptive immune responses [89, 90]. Adaptive immunity provides specific defense tools in vertebrates that are critical for elimination of alloantigens. Cells

of both the innate and adaptive immune system contribute to the generation of substance P and are also targets to this neuropeptide [16, 15]. In the following sections, we discuss how substance P controls the immune responses at multiple levels ranging from recruitment and proliferation to modulation of immune cell activation (Table 1).

Immune cell migration

Substance P is a key player in cellular migration and exerts this function either directly or via induction of a number of chemokines, their receptors, and adhesion molecules. Substance P participates in migration of several innate immune cells, such as neutrophils. For example, neutrophils show an attenuated chemotactic response to exogenous IL-1 β in NK1R-deficient mice [134]. SP can also augment leukocyte recruitment indirectly via inducing the expression of chemokines, such as macrophage inflammatory proteins (MIP-1 β or CCL4 [71], MIP-2 or CXCL2 [49]), monocyte chemoattractant protein-1 (MCP-1 or CCL2) [135, 67], CCL5 [136], and IL-8 [137]. SP stimulates human corneal [137] and epithelial cells [64, 68] as well as neutrophils [138], mast cells [139], and fibroblasts to produce IL-8 (Fig. 2a) [140], a powerful neutrophil chemotactic protein. In activated human T lymphocytes, SP significantly upregulates the expression of MIP-1 β , a β -chemokine that mediates the migration of lymphocytes and monocytes to the site of inflammation. This process is mediated by NF- κ B and is abrogated by the specific NK1R antagonist CP-96,345 [62]. SP can also regulate the movement of dendritic cells toward lymph nodes via modulating the expression of chemokine receptors and adhesion molecules. For example, SP upregulates the expression of chemokine receptor CCR7 [141, 96] as well as the adhesion molecule, macrophage-1 antigen (Mac-1, CD11b/CD18) and its ligand, ICAM-1 (CD54) on NK1R⁺ dendritic cells, all facilitating the direction of these cells toward lymph nodes [95, 142, 96]. Substance P acts as a

Table 1 Immunomodulatory effects of SP as compared to a few other neuropeptides

Neuropeptide	Dendritic cell/macrophage	Lymphocyte			
		Th1	Th2	Treg	Th17
Substance P	+(1)	+(2)			+(3)
NKA	+(4)	+(5)			
CGRP	-(6)	-(7)	+(8)	+(9)	+(10)
a-MSH	-(11)	-(12)	+(13)	+(14)	
VIP	-(15)	-(16)	+(17)	+(18)	+(19)

+ Indicates activation; – denotes inhibition

(1) [91–93], (2) [94, 95, 96], (3) [97, 98], (4) [99], (5) [100], (6) [101, 102, 103, 104, 105, 106, 107, 93], (7) [108, 109, 105, 110], (8) [111, 105, 96], (9) [96], (10) [112], (11)[113–115], (12) [116], (13) [96], (14) [117, 96, 118, 119, 116], (15) [120, 121, 122, 93], (16) [120, 123, 124], (17) [125, 126, 127, 96, 128], (18) [129, 130, 131, 96], (19) [132, 133]

potential determinant of endothelial–leukocyte interactions by inducing the expression of an endothelial–leukocyte adhesion molecule (ELAM-1) by the microvascular endothelium [143]. Immune cell recruitment by SP is fundamental to neurogenic inflammation, which is a critical component of a number of pathologies. For example, substance P recruits leukocytes to the peripheral terminals of nociceptors, where they release neuroactive mediators that contribute to neuropathic pain [144]. In summary, SP appears to play a key role in regulating immune cell migration typically via regulating the expression of chemokines and adhesion molecules.

Immune cell proliferation

Cells proliferate by division into daughter cells, a process that lies at the heart of immunology [145, 146]. Substance P regulates the proliferation of lymphocytes, bone marrow cells, and vascular endothelial cells. Studies show that substance P stimulates human T cell proliferation *in vitro*, probably through upregulation of IL-2 expression [147–151]. NK1R^{-/-} mice have reduced the proliferative response of T cells, suggesting that SP modulates the proliferation of T cells [18]. Human bone marrow mononuclear cells and fibroblasts also proliferate in response to substance P [152]. Furthermore, it has been shown that SP could enhance the proliferation of bone marrow stem cells [153].

The role of nerve-derived SP in hematopoiesis has been supported through its local and systemic effects on NK1R expressing bone marrow stem cells (BMSCs). Substance P exerts a hematopoietic effect through induction of IL-1 and stem cell factor (SCF) in bone marrow stroma [154]. This effect arises from direct contact of SP-generating nerve endings with the bone marrow stroma. Systemically delivered SP may also affect hematopoietic stem cells (HSCs), such as promoting the generation of colony-forming units in the peripheral blood [155].

Substance P targets immune cells to promote angiogenesis [79]. Substance P may regulate angiogenesis directly by inducing endothelial cells to produce nitric oxide [78], or indirectly via its interactions with mast cells and granulocytes. Substance P enhances the expression of vascular endothelial growth factor (VEGF) in mast cells, an action augmented by IL-33 [156], but VEGF blockade does not abolish the proangiogenic property of SP [157]. SP may also modulate angiogenesis by enhancing migration, adhesion, and expression of angiogenic genes by granulocytes. This property of substance P has been exploited to induce angiogenesis at the site of implants [158]. Finally, we note that angiogenesis induced by factors like SP can in turn promote immune response by facilitating trafficking of immune cells to tissues. For instance, in healthy cornea, angiogenesis is actively suppressed which leads to corneal

immune privilege. Breakdown of angiogenic privilege is a major risk factor for corneal transplant rejection. Interestingly, inhibition of NK1R by Lanepitant (an NK1R antagonist) significantly reduces corneal angiogenesis [159].

Immune cell activation

Substance P modulates the activity of innate and adaptive immune cells via a number of intracellular pathways (examples are shown in Fig. 2a). A critical role of substance P lies in its ability to modulate the production of various cytokines by a wide range of immune cells (Table 1). These include cytokines with proinflammatory (e.g., IL-1), immunomodulatory (e.g., IL-10), and chemotactic properties (e.g., IL-8). Cytokines in turn may modulate the effect of substance P; IL-12, IL-18, and TNF α induce NK1R expression in T cells [50, 51], whereas IL-10 and TGF- β prevent NK1R expression [49, 160]. Moreover, IL-1, IL-4, and IFN- γ induce NK1R expression in macrophages [17, 161].

Substance P modulates the activation of various innate immune cells (e.g., phagocytosis and secretion of cytokines) and promotes the survival of innate immune cells such as natural killer cells, macrophages, dendritic cells, neutrophils, mast cells, and eosinophils. Substance P enhances the cytotoxicity of human natural killer cells by upregulating their production of cytotoxic-associated molecules (perforin, granzyme) and natural cytotoxicity receptors (NCR) [162]. SP enhances phagocytosis in both neutrophils and macrophages. In human neutrophils, SP potentiates phagocytosis via stimulating respiratory burst and the production of reactive oxygen intermediates (ROIs) [163, 164]. These processes are fundamental to many pathologies, such as migraine where substance P degranulates dural mast cells [165]. Substance P also enhances phagocytosis in murine peritoneal macrophages via its N-terminus [166], induces oxidative burst, and stimulates synthesis and release of arachidonic acid metabolites (e.g., PGE₂, TXB₂) and toxic oxygen radicals [167, 168]. In addition, substance P enhances the survival of dendritic cells by promoting Bcl-2 expression and subsequent decrease in caspase 3 [94]. In human and murine mast cells, SP induces degranulation and subsequent release of histamine and serotonin [6] and upregulates Toll-like receptor (TLR)-2, thereby promoting its activation [169]. Moreover, SP increases the expression of corticotropin-releasing hormone receptor-1 (CRHR-1) by human mast cells and the activation of this receptor leads to secretion of IL-8, TNF- α , and VEGF, while CRH itself induces the expression of the truncated NK1R (NK1R-T) [170]. Finally, SP stimulates activation, degranulation, and release of O₂⁻ and TXB₂ from eosinophils [171].

Substance P also plays a critical role in the activation of adaptive immune cells. SP enhances immunoglobulin

secretion in murine Peyer's patches, splenic lymphocytes and mesenteric lymph nodes in an isotype-specific manner (particularly, IgA). In certain B cell lymphoma clones, substance P directly stimulates the secretion of IgA, but not of IgM. However, in the presence of lipopolysaccharide (LPS), substance P stimulates a threefold increase in IgM secretion [172], indicating a cross talk between NK1R and TLR4. Depletion of substance P in rodents by capsaicin administration or treatment with a substance P antagonist decreases the number of antibody-secreting cells [173].

The role of substance P in T cell activation and differentiation of helper T cell subsets has been investigated in several studies, although few of them achieved remarkable results. For example, during the inflammatory response caused by murine schistosomiasis, SP binds to NK1R on T cells and induces the production of IFN- γ , a Th1 signature cytokine, while it has no effect on the secretion of Th2 cytokines, such as IL-4 and IL-5 [36]. In a murine colitis model, it was shown that expression of NK1R in mucosal T cells in IL-10-deficient mice was associated with Th1-mediated intestinal inflammation [160]. In addition, via binding to NK1R on DCs, SP can induce a Th1 and T cytotoxic (CTL)-1 bias of effector T cells in mice [79]. In another murine study, NK1R signaling in DCs caused an inhibition of IL-10 synthesis and secretion without affecting IL-12 production. However, after cutaneous administration of these NK1R-signaled DCs, IL-12 was upregulated in host DCs resulting in a remarkable Th1 immune response [78]. Regarding Th17 immunity, one study showed that SP promoted the generation of human memory Th17 cells from non-Th17-committed CD4⁺ memory T cells, but not from naïve CD4⁺ T cells. They also showed that the other members of the tachykinin family, neurokinins A and B, had no effect on the differentiation of naïve and memory T cells [98]. Finally, administration of substance P during the primary immune response amplifies the secondary immune response by activating CD8⁺ T lymphocytes [174].

Substance P affects the immunomodulatory capacity of mesenchymal stem cells (MSC). Evidence suggests that SP treatment may recover the immunosuppressive function of late passage MSCs by potentiating their ability to secrete TGF- β 1, which can enhance the therapeutic activity of ex vivo expanded MSCs in long-term culture. Using an NK1R antagonist, the restoration of the weakened activity of MSCs could be abolished [175].

It is important to note that the two NK1 receptor isoforms mediate different immunological effects when activated by SP [56]. For example, SP upregulates NF- κ B and IL-8, and stimulates PKC δ via NK1R-F, but not NK1R-T in certain human cells. There are also differences in the timing of SP-induced ERK activation in cells expressing the two different forms of the receptor. ERK activation via NK1R-F is more

rapid than via NK1R-T. These data suggest the role of the carboxyl terminus of NK1R in the activation of downstream signaling pathways [30]. On the other hand, Chernova et al. reported that human peripheral blood monocytes express NK1R-T, but not NK1R-F; however, SP interactions with NK1R-T do not lead to calcium (Ca²⁺) mobilization. When NK1R-F is transfected into monocytes, SP can mobilize Ca²⁺. Furthermore, via NK1R-T, SP can enhance the CCR5 ligand CCL5-elicited Ca²⁺ mobilization leading to chemotaxis, indicating that even the NK1R-T can be functional in monocytes. From these results, we can conclude that in human monocytes, NK1R-T activates selected signaling pathways and mediates chemotaxis [136]. Concurrently, Lai et al. showed that a human monocyte/macrophage cell line expresses only NK1R-T, which cannot trigger a Ca²⁺ response upon addition of SP, although SP increases the CCR5-preferring ligand RANTES (CCL5)-mediated Ca²⁺ increase in these cells. When these cells differentiate to a macrophage-like phenotype, they express NK1R-F, which lead to an SP (10⁻⁶ M)-induced Ca²⁺ response [29]. Taken together, both truncated and full-length NK1R are functionally active in innate immune cells and their functional roles seemingly depend on the cell type and may differ between primary cells and cell lines.

Immunopharmacology of substance P

The list of NK1R antagonists (NKAs) is growing. NKAs are of two types: peptide antagonists and non-peptide antagonists. An example of the former group is spantide, a therapeutic peptide which can treat corneal infection [176]. The most well-known non-peptide antagonist is aprepitant, a morpholine derivative used as an antiemetic, antidepressant, anxiolytic, and antitumor drug [177–180]. Several studies have reported the effect of NKAs on innate and adaptive immune responses, including the modulation of cytokine production, immune cell proliferation, and immune cell migration. Spantide I, for example, enhances proinflammatory (e.g., IL-1 β and TNF- α) and anti-inflammatory cytokines (e.g., IL-10) and suppresses Th1-associated cytokines (e.g., IL-18, IL-12 and INF γ) [176]. Spantide suppresses IL-2 production by human and murine T cells and thus may suppress T cell proliferation [150]. Spantide antagonizes the role of SP in immune cell recruitment; for example, it decreases the influx of neutrophils and CD4⁺ T cells in the corneal lesions. When treated with spantide I, corneal IL-6 and CCL3 production was reduced in animal models [181]. Similar functions have been reported for other NK1R antagonists as well. Lanepitant suppresses leukocyte infiltration, and lymph- and hemangiogenesis in the cornea [159]. Spantide also suppresses secretion of immunoglobulins from B cells in rats [173]. Finally, NKAs can suppress hemangiogenesis

by decreasing stem cell factor (SCF), IL-1, IL-3, and GM-CSF [182, 154]. Taken together, these results indicate that major advances in our understanding of the mechanism of action of SP have emerged in the past decade via pharmacologic blockade of SP in animal models.

There are few clinical trials using the SP antagonist, aprepitant, in HIV-infected patients. In a clinical trial conducted by Pablo Tebas, Steven D. Douglas and colleagues in HIV-infected patients, aprepitant did not show significant antiviral activity, although aprepitant-treated patients showed decreased numbers of CD4+ PD1+ T cells, so-called exhausted T cells, and decreased plasma levels of substance P and soluble CD163, suggesting that blockade of the NK1R pathway plays a role in regulating monocyte activation in HIV infection [183].

Immunomodulatory role of the substance P in disease

In this section, we briefly discuss the clinical implications of SP immunobiology for neurology and ophthalmology, with particular focus on SP and ocular surface diseases, where neuropeptide research is on the rise (see Table 2).

Neurologic diseases

The immunomodulatory role of SP in neurologic conditions has been widely recognized. Substance P exerts its immunomodulatory role within the central nervous system (CNS) through its ability to bias the inflammatory response toward Th17 immunity, as well as to regulate Th1/Th2 balance toward either Th1 or Th2 response depending on the nature of antigens [98, 206]. In addition to regulating Th1 and Th2 cells, SP can bias the inflammatory response toward Th17 immunity [98], which is implicated in the pathogenesis of multiple sclerosis/experimental autoimmune encephalomyelitis (EAE). In this way, SP may contribute to the maintenance of CNS inflammation during the chronic phase of EAE [201]. NK1R antagonists, such as CP-96,345, stabilize the blood–brain barrier and down-regulate Th1-type cytokines [200].

SP has a well-recognized role in nociceptive neurotransmission [70]. SP may recruit CNS glial cells under pathological pain conditions [207]. For example, bone fracture pain is associated with increased expression of glial activation markers, which can be attenuated with an NK1R antagonist [208]. It is not yet clear whether these

Table 2 Immunomodulatory role of the substance P: lessons from disease models

Disease model	Administration of SP	Blockage of NK1R/SP/NEP system: knockout (KO) or pharmacological blockade (PB)	References
Arthritis	Proinflammatory	Anti-inflammatory (KO)	[184, 185]
Type 1 diabetes	Reversal of diabetes (Intrapancreatic in NOD mice)		[186]
Inflammatory bowel disease		Anti-inflammatory (KN, PB)	[187–190]
Colitis		Anti-inflammatory (PB)	[191, 160]
Psoriasis		Anti-inflammatory (PB)	[192]
Contact dermatitis		Anti-inflammatory (PB, KO)	[193, 194]
Asthma	Proinflammatory (Intranasal)	Anti-inflammatory (PB)	[195–197]
Immune mediated liver disease		Anti-inflammatory (PB)	[198]
Lung injury in sepsis		Anti-inflammatory (PB)	[199]
Experimental Autoimmune Encephalitis (EAE)		Anti-inflammatory (PB, KO)	[200, 201]
Enterocolitis		Anti-inflammatory (KO)	[202]
<i>Salmonella enterica</i> infection		Protective through promoting IgA generation (KO)	[203–204]
		Increased susceptibility through suppression of IL-12 and IFN- γ (PB)	
<i>Pseudomonas aeruginosa</i> corneal infection	Proinflammatory (Intraperitoneal)	Increased resistance in susceptible mice (PB)	[205]
		Increased susceptibility in resistant mice (PB)	
HSV-1 corneal infection		Anti-inflammatory (PB)	[181]
Corneal Neovascularization-suture model		Anti-inflammatory (PB)	[159]
Corneal Neovascularization-alkali burn model		Anti-inflammatory (PB)	[159]

effects are direct, as inhibition of nitric oxide can attenuate the proinflammatory effects of SP [209]. Given the ability of microglia and astrocytes to oppose opioid analgesia [210], it is notable that co-administration of an NK1R antagonist with morphine for 6 days attenuates hyperalgesia and expression of glial activation markers [211].

In Parkinson's disease, CNS immune cells such as microglia and astrocytes are activated and release proinflammatory cytokines [212–215]. Substance P has been found to mediate this inflammatory response, which can be suppressed using NK1R antagonists. These observations suggest the possible use of NK1R antagonists for neuroprotection in Parkinson's disease [215]. Finally, SP can enhance the blood–brain barrier permeability by disrupting the tight junction proteins [216], which facilitates edema (leading to increased pressure in the cranium or spinal canal) following traumatic brain injury, spinal cord injury, and stroke [217]. Accordingly, NK1Rs are therapeutic targets for these conditions [218].

Ocular diseases

Neuropeptides are generated by a number of cells in the eye. For example, sensory nerves of the eye generate substance P, while vasoactive intestinal polypeptide and neuropeptide Y are secreted by parasympathetic and sympathetic innervations of the eye, respectively [219, 220]. In the retina, the principal SP localizations are amacrine cells in the proximal inner nuclear layer and displaced amacrine cells in the ganglion cell layer [221–223]. Involvement of SP has been described in a few inflammatory and pathological conditions of the retina. Amacrine-derived SP has been reported to be involved in the loss of immune privilege post-retinal laser burn through its potential to promote an inflammatory environment that in turn activates resident macrophages and microglia. NK1R antagonists could prevent or curb inflammation post-ocular trauma [224]. On the other hand, there are reports on anti-inflammatory effects of SP in the retina. SP can reportedly prevent laser-induced retinal degeneration *in vivo*, by suppressing inflammation and reducing neovascularization. Its anti-inflammatory effects are exerted via reduction of TNF- α and increase of IL-10; the anti-angiogenic effects are achieved via downregulation of CD31+ retinal vessels [225].

Substance P plays significant roles in corneal biology and diseases. A number of sources have been identified for corneal SP, namely trigeminal sensory neurons, corneal epithelial cells, stromal keratocytes, and immune cells [15, 13]. Several reports have explored the role of SP in maintaining corneal epithelial integrity and regulating regeneration of the corneal epithelium. The corneal epithelium is the first barrier against bacteria and other foreign antigens, and actively interacts with

other corneal cells, such as resident immune cells and corneal nerves [226, 15]. Substance P targets corneal epithelial cells via binding to the NK1R receptor. Substance P contributes to the maintenance of tight junctions in human corneal epithelial cells by upregulating the tight junction proteins, E-cadherin [227] and zonula occludens (ZO-1) [228]. Corneal epithelial cells also respond to SP by increased synthesis of IL-8 and MIP-2, which in turn lead to the recruitment of neutrophils [137]. Substance P functions as an important modulator of corneal (epithelial) wound healing by affecting the process of corneal epithelial migration via enhancing the effect of migrating–promoting agents and modulating epithelial cell attachment to the extracellular matrix. The synergistic effect of SP and insulin-like growth factor (IGF)-1 has recently been reported, yet studies are needed to elucidate the exact underlying mechanism. Substance P likely promotes corneal epithelial cell migration by IGF-1, fibronectin, and IL-6. This sensitization is likely mediated by a signaling pathway triggered by NK1R, one that leads to the activation of phospholipase C, the IP3-mediated release of Ca²⁺ from the endoplasmic reticulum (ER), and the activation of calmodulin-dependent protein kinase II (CaM-PK II). In turn, the target proteins of CaM-PK II may stimulate signaling pathways activated by IGF-1 (or its C domain), fibronectin, or IL-6 [229]. Additionally, SP and IGF-1 stimulate the attachment of corneal epithelial cells to various extracellular matrix proteins by upregulating $\alpha 5\beta 1$ integrin (fibronectin receptor/integrins function as cell surface receptors for fibronectin, an adhesion glycoprotein which provides a temporary matrix for epithelial migration) and inducing tyrosine phosphorylation of focal adhesion kinase and paxillin in corneal epithelial cells [230, 231]. This synergistic effect of SP and IGF-1 improves healing of corneal epithelial wounds and defects in individuals with neurotrophic and anhidrotic keratopathy, Riley–Day syndrome, herpetic keratitis, and in rats with diabetic keratopathy [232–235]. Even though the importance of SP in corneal wound healing has been suggested, it was mainly explored in the context of the local effect of SP on accelerated wound healing. SP may also act systemically, recruiting CD29+ stromal-like cells from the periphery to the site of injury, resulting in accelerated wound healing [155].

By modulating the balance between pro- and anti-inflammatory cytokines, SP may affect susceptibility to corneal infections. Substance P has been shown to downregulate the mTOR pathway, leading to decreased expression of the anti-inflammatory cytokine IL-10 and upregulation of the proinflammatory cytokines IL-12p40 and IL-23 (Fig. 2). In animal models, this change results in increased susceptibility to corneal infection [61, 236]. In contrast, SP regulates the IFN- γ production of natural killer cells and thus protects against bacterial infection. Treatment with SP antagonist spantide I significantly decreases corneal IFN- γ and IL-18 protein levels, which leads to

corneal infection and perforation. IL-18 upregulates IFN- γ production by natural killer cells through IL-12 independent mechanisms and exerts a protective role in the cornea of infected mice, such as after *Pseudomonas aeruginosa* infection [237].

Substance P directly stimulates corneal neovascularization, an effect which can be blocked by NK1R antagonists [159]. Normal cornea is known to have angiogenic and immune privilege. Breaking angiogenic privilege can lead to disruption of immune privilege as well [238]. An increased SP level, which is seen in inflammation [239, 83], could challenge the privilege by stimulating endothelial cell proliferation. Loss of angiogenic privilege has clinical significance in corneal graft rejection [240].

The cornea constitutes one of the most densely innervated tissues in the human body [241, 242] and corneal nerve density and function are affected in a number of corneal pathologies such as dry eye disease [243–249]. In healthy cornea, SP is generated by corneal nerves and epithelium and serves to protect epithelial integrity [220, 250, 251, 229]. In dry eye disease, desiccating stress disrupts the epithelial integrity and induces nerve loss [252]. One expects that substance P production increases initially by the stressed epithelium and nerve fibers as a defensive mechanism to protect the epithelial integrity. SP then recruits immune cells via enhancing the production of chemokines, such as IL-8. As the disease progresses, nerve and epithelial loss expectedly lead to reduced production of SP, while inflammation results in higher production of SP by the residents and recruited immune cells. In other words, a reduction in nerve density, such as in the chronic phase of dry eye disease, would expectedly lead to a reduction in SP level. In contrast, inflamed nerve cells, like those seen in early dry eye disease, would expectedly generate higher levels of SP. Corneal transplantation could be another interesting condition: Here, we expect a sudden release of copious amounts of SP in the graft bed along with an immediate reduction in the production of nerve-derived SP in the grafted cornea. Other sources, such as macrophages, might produce more SP at the later stages. In high-risk setting (HR), where inflammation exists prior to transplantation [253], SP level is likely higher than normal even before transplantation. SP generation likely increases with the progression of alloimmune response and leads to increased angiogenesis and graft rejection. These hypotheses, however, remain to be tested in specific disease contexts.

Concluding remarks

Substance P represents an important component of the immune response. Despite the insight that has been gained from in vitro and in vivo studies, the several unresolved

questions related to the role of SP in immunobiology remain to be elucidated. For example, the relationship between SP receptor levels of expression in different cell types and diseases is still unknown. This presents a challenge when interpreting clinical studies, where NK1R antagonists may need to be stratified to appropriate patient subsets. We need model systems that are simple enough to allow elucidation of the roles and responses of different cell types and their environment that will help elucidate the role of SP in interactions between nerve, immune, vascular cells, as well as connective tissue cells. The cornea is potentially an excellent model system for such studies, as it carries the highest density of nerve cells and has proven to be an invaluable model system for immunology and angiogenesis. The clinical implications of such studies are potentially significant. Cornea transplants, for instance, are the most common human tissue transplants in the USA. It is likely that several studies related to substance P immunobiology using ocular surface disease models will emerge in the years to come.

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