Arch Immunol Ther Exp, 2004, 52, 338–347
PL ISSN 0004-069X

WWW.AITE–ONLINE.ORG
Review

Received: 2004.05.21
Accepted: 2004.07.20
Published: 2004.10.15

Neurokinin receptors: relevance to the emerging immune system

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Source of support: grant CA-89868 awarded by the National Cancer Institute.

Summary

The adult bone marrow (BM) is the major site of the emerging immune system. Hematopoiesis is the process whereby immune cells are generated from a finite number of hematopoietic stem cells. Hematopoiesis is regulated by soluble mediators and intercellular interactions. A major regulatory mechanism of hematopoiesis involves bidirectional crosstalk with the neural system. This communication mainly occurs by the release of neurotransmitters from innervated fibers. The neurotransmitters interact with specific receptors on BM resident cells and release other hematopoietic regulators such as cytokines. Together, the neurotransmitters and cytokines form a complex network to regulate hematopoiesis. Among BM resident cells, the stromal cells are particularly relevant for two reasons: 1) they represent non-neural sources of neurotransmitters, and 2) stromal cells express specific receptors for neurotransmitters. This review focuses on the hematopoietic effects of neurotransmitters belonging to the tachykinins. The two major tachykinins focused in this review are substance P and neurokinin (NK)-A, 11 and 10 amino acid peptides. In BM, the tachykinins interact with two major NK receptors: NK-1 and NK-2. These two receptors appear to limit tachykinin-mediated effects on hematopoiesis. The central roles of NK receptors within a network comprising of cytokines and tachykinins are reviewed.

Key words: **neurokinin • substance P • cytokines • neuropeptides • hematopoiesis**

Abbreviations: **NK** – neurokinin, **NK-R** – neurokinin receptor, **PPT-1** – preprotachykinin 1, **HSC** – hematopoietic stem cell, **MSC** – mesenchymal stem cell, **BM** – bone marrow, **HK-1** – hematokinin 1, **SP** – substance P, **CMP** – ommon myeloid progenitor, **CLP** – common lymphoid progenitor.

Full-text PDF: http://www.aite-online/pdf/vol_52/no_5/6340.pdf

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HEMATOPOIESIS

The adult bone marrow (BM) is host to at least two stem cells: the hematopoietic (HSC) and the mesenchymal (MSC)[11, 78]. While the HSC is the prototype stem cell, and also the most studied, the understanding of the MSC is at its initial stage. The major functions of HSCs are to replenish the adult immune/blood system throughout life by the process of hematopoiesis[78]. HSCs exhibit classical properties of stem cells by their self-renewal ability and commitment into two major lineages, the common lymphoid (CLP) and common myeloid progenitors (CMP). CLPs branch into lineages that generate T and B cells, whereas CMPs differentiate into lineages that form erythrocytes, granulocytes, and platelets[33–35, 78]. Natural killer and dendritic cells are commonly produced by both CLPs and CMPs[33, 78]. Early thymic progenitors migrate from the BM to repopulate the thymus[13]. The preceding argument supports the concept that the BM is the primary organ of the emerging immune system.

HSCs are found in areas close to the endosteum in regions of low oxygen, away from the main sinusoid of the BM[28]. Anatomically, HSCs are in contact with BM stromal cells[37]. The close association between HSCs and the BM stroma allows the stromal cells to produce supporting molecules for the HSCs[37]. MSCs surround the inner region of blood vessels within the BM[16]. Despite the anatomical distance between HSCs and MSCs, these two stem cells are functionally connected, since MSCs are the source of the supporting stromal cells[4, 14].

Cells within the BM microenvironment respond to different types of stimuli to support hematopoiesis[27]. Cellular responses cause production of soluble factors such as cytokines, chemokines, neurotrophic factors, and neuropeptides[23]. The BM is innervated with peptidergic and sympathetic fibers[19, 67, 68, 76]. This suggests that the functions and, perhaps, properties of HSCs could be influenced by the neural system. In fact, different neurotransmitters have been shown to exhibit regulatory functions on hematopoiesis[20, 23]. Association with a particular neurotransmitter is not mutually exclusive of other factors within the BM microenvironment[52]. For example, the neurotransmitter substance P (SP) interacts with several other cytokines, extracellular matrix proteins, and endopeptidases to attain hematopoietic homeostasis[52]. In short, homeostasis in the hematopoietic/emerging immune system is attained by multiple intracellular pathways triggered by receptors and their respective ligands. This review focuses on the role of neurokinin receptors (NK-Rs) in hematopoiesis. NK-Rs are the natural receptors for neurotransmitters belonging to the tachykinin family of peptides[45, 50].

NEUROENDOCRINE-IMMUNE/HEMATOPOIETIC AXIS

Cytokines have been considered the prototype regulators of hematopoiesis. However, other molecules, such as neurotransmitters, are emerging as another category of hematopoietic regulators[23]. Grouping of hematopoietic regulators is only academic, since members within each group exhibit overlapping functions. Studies on mechanisms by which the neural system influences secondary lymphoid organs and the thymus indicate that there are overlaps between the neural and immune systems to form a neural-immune axis[18, 23, 38]. Further studies on the hematopoietic system led to a modified neural-immune/hematopoietic axis[57].

Tachykinin-positive nerve fibers are found within the BM[19, 21, 22, 67, 76, 69]. The anatomical link that forms part of the neuroendocrine-immune/hematopoietic axis reinforces the concept that multiple systems are involved in hematopoiesis. In addition to neurotransmitter release in the BM, the neural system could also influence hematopoiesis through the release of neurohormones derived from the hypothalamus-pituitary/adrenal axis[32].

Immunohistochemical staining for particular neurotransmitters in synaptic vesicles strongly suggests that the release of these neurotransmitters might be a major mechanism by which the neural system affects hematopoiesis[19, 22]. However, other research studies show that innervation of the BM might be important for the retention of HSCs and progenitors in the BM[1, 2, 71]. Denervation of fibers entering the BM led to depletion of BM cellularity[1]. Other human studies show that surgical trauma with head injuries led to disruption of hematopoiesis and redistribution of hematopoietic progenitors[2, 3, 71, 72]. Lowered hematopoiesis has been reported in BM areas below spinal cord injuries (SCI)[25]. We now show increased progenitors of granulocytes-monocytes and erythrocytes in patients with SCI (Table 1). Although the results shown in Table 1 do not indicate the tissue sources of the circulating progenitors, we can extrapolate a hypothesis based on information in the literature. Hematopoietic progenitors in SCI could be derived from sites of extramedullary hematopoiesis, based on previous studies showing the loss of cellularity in nerve injury to the BM[25]. SCI could be accompanied by psychological stress, which is linked to the presence of increased levels of glucocorticoids[77]. Since the latter could cause redistribution of immune cells, we hypothesize that in SCI a similar re-

Table 1. BM progenitor colonies in peripheral blood from subjects with spinal cord injury (SCI)

Subjects	CFU-GM	BFU-E	CFU-E
SCI	30 ± 5	36 ± 4	< 1
Healthy controls	10 ± 4	(2–5)	12 ± 3

Peripheral blood was obtained from patients at East Orange Veteran's Hospital, East Orange, NJ, USA. Mononuclear cells were separated from peripheral blood by Ficoll Hypaque density gradient. Mononuclear cells (2×10^5) were assayed for the following progenitors: granulocytic-monocytic (CFU-GM), early erythroid (BFU-E), and late erythroid (CFU-E), as described[26]. Analyses were done with clonogenic assays performed in methycellulose matrix as described. The data are presented as the mean ± SD, n = 10 different patients. Each patient was tested in parallel with age- and sex-matched healthy controls.

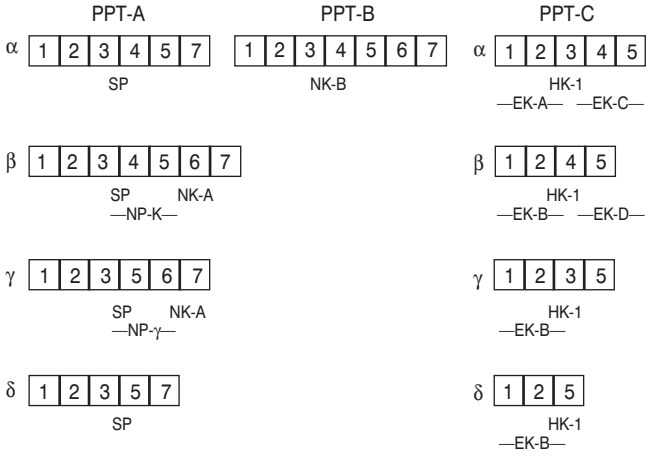

Figure 1. Transcripts for the three PPT genes and the major peptides produced by each transcript are depicted[9, 47]. Explanations: HK-1 – hemokinin 1, EK-A – endokinins A, SP – substance P, NP-K – neropeptide K.

-localization of hematopoietic progenitors might be operative. Hematopoietic dysfunctions were observed in a model of epilepsy in which the brain is directly stimulated[10], suggesting that similar or related mechanisms might be occurring in SCI. Thus it is not surprising that in otherwise healthy individuals, traumatic injuries with head involvement led to altered hematopoiesis even prior to inflammation[2]. The contents of this section have direct impact on the immune system, since neural alteration of hematopoiesis translates into immune dysfunction.

TACHYKININS

The preprotachykinin (PPT) gene encodes neuropeptides that belong to the tachykinin family[8]. The tachykinins are mostly 10- to 12-amino-acid peptides that share common carboxyl termini, Phe-X-Gly--Leu-Met-NH_2, where X is either an aromatic or branched aliphatic residue[47]. PPT-1 encodes two of the most studied tachykinins, SP and neurokinin (NK)-A[47]. SP and NK-A are 11- and 10-amino-acid peptides, respectively. PPT-A comprises 7 exons, which can be alternately spliced and modified to form 4 transcripts: α-, β-, γ-, and δ-PPT-1 (Fig. 1). SP is encoded by exon 3, present in each transcript, and NK-A is encoded by exon 6, present in two transcripts, β and γ[47]. NK-K and neuropeptide γ are also biologically active peptides that are encoded by exons 3, 4, 5, and 6 of β-PPT-1 and exons 3, 5, and 6 of γ-PPT-1, respectively[15, 47]. The PPT-A gene is expressed in neural (peripheral and central) and non--neural tissues, BM, and immune cells[57, 61].

Two other PPT genes have been reported: PPT-B and PPT-C[47, 75]. The PPT-B gene has 7 exons, with exon 5 encoding NK-B[8]. PPT-B is expressed in the brain and in peripheral tissues[47]. The PPT-C gene produces hemokinin 1 (HK-1), expressed in hematopoietic cells[36, 75, 76]. Unlike PPT-A and PPT-B, the PPT-C gene has 5 exons. Despite this difference, the PPT-C gene is alternately spliced to produce 4 transcripts, α-, β-, γ-, and δ-PPT-C[43, 47]. It now appears that HK-1 is distinctively expressed outside the neural system. HK-1 is encoded by exon 2, found in each transcript[47]. Four different peptides, endokinins A, B, C, and D, are also produced by the PPT-C gene[43, 47].

NK-RS AND SUBTYPES

The tachykinins interact with three natural NK-Rs: NK-1, NK-2, and NK-3[23]. The NK-Rs belong to the family of 7-transmembrane, G-protein-coupled receptors[8]. Although the tachykinins can bind with varying affinities to a specific NK-R subtype, each peptide shows binding preference for a particular NK-R subtype. For example, SP exhibits binding preference for NK-1, whereas NK-K, neuropeptide γ, and NK-A show binding preferences for NK-2[17, 47]. The tachykinins can, however, interact with weak binding affinity to other NK-Rs[9]. HK-1, endokinins A and B, and SP share the NK-1 receptor[36, 43, 75, 76]. Endokinins C and D interact weakly with the NK-Rs, suggesting that there might be unidentified natural receptor for these two peptides[43]. NK-Rs are widely expressed in neural and non-neural systems. BM stroma, immune, and hematopoietic cells also express NK-Rs[8, 23]. SP binding sites are also present in hematopoietic progenitors[23, 30].

TACHYKININS AND HEMATOPOIESIS

There are two sources of SP and NK-A in the BM, as neurotransmitters and from resident BM cells[56]. Studies on the roles of SP and NK-A in hematopoiesis indicate that both peptides affect hematopoiesis at

multiple stages within the hematopoietic hierarchy. Figure 2 depicts the role of SP, fragments of SP, and NK-A on the different developmental stages of hematopoiesis[23]. Arrows represent hematopoietic stimulation by SP. At each point of the SP effect, NK-A acts as a negative feedback by exerting opposing hematopoietic effects[54]. The hematopoietic effects of SP and NK-A are mostly indirect, through stimulation of BM stroma. SP and NK-A induce the production of cytokines with stimulatory or inhibitory effects, respectively[58]. The effects of SP on hematopoiesis are not limited to the myeloid compartment (Fig. 2). SP acts as a co-stimulator during the late stage of B cell maturation[44] and has been implicated in the development of T cells in the thymus[62].

Although SP interacts with NK-1 to exert stimulatory hematopoietic effects, this receptor could also exhibit hematopoietic suppression when it interacts with the amino terminal fragment of substance P, $SP_{(1-4)}$[26]. Also, in cases in which signaling by NK-1 is blocked, SP interacts with NK-2 to exhibit hematopoietic suppression[5]. Thus, the hematopoietic effects of tachykinins are determined by the subtype of interacting NK-R. Regardless of the mechanism, the functions of tachykinins on hematopoietic immune functions involve networks formed by cytokines and intracellular crosstalk between NK-1 and NK-2[5, 61, 63]. Ultimately, the normal asynchronous pattern of hematopoietic activities is attained to maintain homeostasis in the BM[27, 48].

The hematopoietic effects mediated by SP and NK-A correlate with the cytokines that each peptide produced in BM cells[23]. The negative effects of NK-A on the proliferation of BM progenitors suggest that NK-A might be protective to HSCs[55]. A protective role for NK-A is construed based on the predominant types of PPT-1 transcripts found in normal BM cells and in leukemia cells. Normal BM stromal cells express β-PPT-1, while leukemic cells express only α-PPT-1[60]. While β-PPT-1 is capable of producing

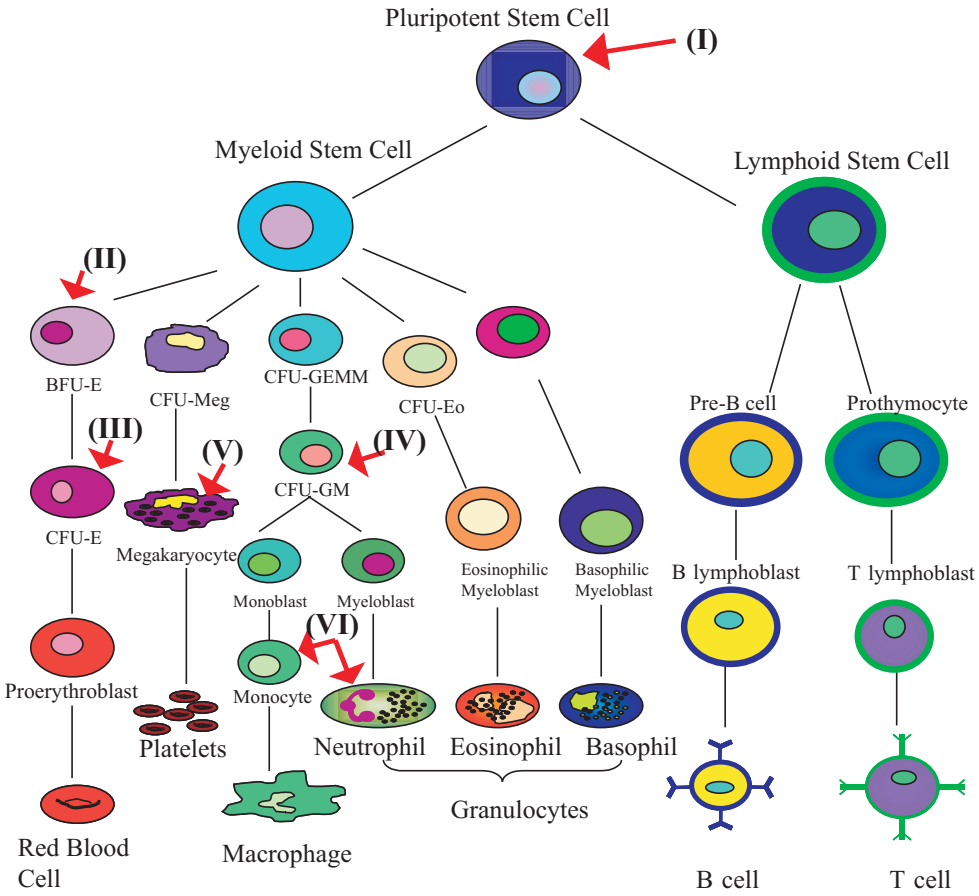

Figure 2. Summary of the hematopoietic effects exerted by SP, NK-A, and SP fragments. **I** – SP mediates stimulatory effects on primitive BM progenitors[52]; $SP_{(1-4)}$ blunts BM progenitor proliferation[26]. **II** – SP and $SP_{(4-11)}$ stimulates the proliferation of early (BFU-E) and late (CFU-E) erythroid progenitors[52]. **III** – SP synergizes with cytokines (e.g. IL-3, SCF, GM-CSF) to induce the proliferation of mixed myeloid-erythroid colonies, CFU-GEMM[23]. **IV** – the effects of CFU-GM by SP are opposite to those by NK-A and $SP_{(1-4)}$: SP exerts hematopoietic stimulation[53]; NK-A and $SP_{(1-4)}$ mediates inhibitory/blunting effects[26]. Arrows respresent hematopoietic stimulation by SP.

both NK-A and SP, α-PPT-1 can only produce SP (Fig. 1). In normal hematopoiesis, SP and NK-A, through the production of distinct cytokines, exert opposite effects with respect to the proliferation of hematopoietic progenitors (Fig. 2). This suggests that SP and NK-A might be able to regulate the proliferation of HSC through autocrine and/or paracrine mechanism. Such a regulatory mechanism might not be possible in leukemic cells, which produce only SP[39, 40, 60]. This argument is supported by reports showing an autocrine role for SP on the proliferation of basophilic leukemia cells[66].

We have not detected NK-3 on human hematopoietic progenitors or BM stromal cells (unpublished observation). However, NK-3 has been demonstrated on immune cells and a stromal cell line[23, 24]. Since immune cells are differentiated BM progenitors, the disparity in NK-3 expression on hematopoietic progenitors and immune cells might be explained by NK-3 being linked to cell differentiation.

HK-1 has been implicated in hematopoiesis[75]. A link between HK-1 and tachykinins derived from the PPT--A gene is currently unclear. HK-1 regulates B and T lymphopoiesis[75, 77] and directly affects the transition from pro-B to pre-B cells[75]. HK-1 promotes the survival and expansion of B cell lineage[13, 31, 35] and similarly facilitates T cell development at specific stages[68].

The regulation of the PPT-1 gene and/or functions of PPT-1 peptides could be altered, leading to BM disruption. Since the innate and adaptive immune systems depend on proper functioning of the hematopoietic system, dysregulation of HSCs would have direct impact on immunity. One or perhaps all of the PPT-1 peptides involved in the regulation of hematopoiesis might be implicated in hematological deficiencies and in inflammatory responses[12, 60]. Indeed, SP is involved in both stem-cell disorders and in immunity to pathogens[70]. Although not demonstrated, the inhibitory effect of NK-A could be important to BM failure. Thus, a full understanding of the mechanisms by which the PPT gene is regulated would lead to in--depth comprehension of hematopoietic modulation.

SP has been shown to be involved in hemorrhagic shock, a time at which there is an acute need for the replacement of blood and immune cells[49, 51]. During hemorrhagic shock, SP exhibits functional pleiotropism so as to maintain a balance in hematopoiesis. Hypoxia, which is linked to hemorrhagic shock, activates the transcription factor hypoxia-inducible factor 1α, which interacts with the PPT-1 promoter to induce its expression[49]. The induction of SP stimulates hematopoiesis so as to replace immune and blood cells[49, 51]. During the period of hemorrhagic shock, SP also acts as an anti-apoptotic factor so as to protect BM cells from the insults caused by acute lowering of oxygen in the BM[49].

TACHYKININS AND CYTOKINES IN HEMATOPOIESIS

Cytokines linked to the hematopoietic functions of SP include interleukin (IL)-1, IL-3, granulocyte-macrophage colony-stimulating (GM-CSF), and stem-cell factor (SCF)[61]. SP induces the production of these cytokines, which exhibit stimulatory effects on hematopoiesis. Alternatively, the cytokines induced by SP could activate BM cells through an autocrine and/or paracrine mechanism to produce other cytokines with hematopoiesis-stimulatory effects[52]. For example, SP induces the production of IL-1, which stimulates the induction of hematopoietic factors with direct and indirect effects on HSCs[52].

In contrast to SP, the hematopoietic effects of NK-A could be stimulatory or inhibitory, depending on the particular hematopoietic lineage[53, 55]. NK-A inhibits the proliferation of granulocyte-monocyte progenitors, but stimulates erythrocytic progenitors[55]. The negative functions of NK-A can be explained by the production of hematopoietic suppressors, MIP-1α and transforming growth factor β (TGF-β)[61].

NK-Rs in hematopoiesis

The NK-Rs mediate hematopoietic functions within a network of soluble factors such as cytokines[23, 57]. The regulation of hematopoietic functions by NK-1 and NK-2 involves distinct and overlapping intracellular signaling pathways. This section discusses a network comprising cytokines and tachykinins, with NK-Rs placed at the center. Discussions will include crosstalk between NK-1 and NK-2 as a major method by which the NK-Rs maintain hematopoietic homeostasis.

NK-1 show tissue-specific regulation, with constitutive expression in neural cells and the ability to be inducible in BM stroma[7]. NK-1 and NK-2 exhibit a yin-yang relationship with respect to their expression in BM stroma[5, 61]. Currently it is unclear if other cells show similar regulation. In BM stroma, while NK-2 is expressed at high densities, NK-1 expression is downregulated, and *vice versa*[5]. The opposing expressions of NK-1 and NK-2 are consistent with the hematopoietic functions associated with these two NK subtypes[5]. Interactions between NK-2 and its preferred ligand, NK-A, lead to inhibitory effects on the proliferation of BM progenitors[55]. In contrast, binding of SP to NK-1 mediates an increase in the proliferation of BM progenitors[53]. Thus, in the event

that a situation requires negative hematopoiesis, interactions between NK-2 and NK-A would be operative. To obtain effective hematopoietic suppression, the microenvironment in the BM would require low effects of NK-1, which could be achieved if NK-1 expression is downregulated.

The opposite expressions of NK-1 and NK-2 could be partly explained at the level of gene transcription (Fig. 3). SP/NK-1 interactions lead to the production of cytokines, such as GM-CSF and SCF. Either directly or indirectly, nuclear factor κB is activated and translocated to the nucleus to activate the NK-1 promoter[7, 65]. The cytokines reported as activators of NK-1 are linked to cell-cycle transition, and might therefore explain why SP mediates increased proliferation of hematopoietic progenitors[52]. Cytokines that are associated with NK-1 activation cannot activate the NK-2 promoter[5]. This is demonstrated by TGF-β being able to induce NK-2 expression while downregulating the expression of NK-1[5]. Thus, at the level of the genes, it is expected that NK-1 expression concomitantly downregulates the expression of NK--2. Given these arguments it is not surprising that NK-2 is highly expressed in unstimulated BM cells, whereas NK-1 expression is low to undetectable[5, 57].

Based on the above discussion, it is expected that the role of NK-1 should be linked to hematopoietic stimulation. However, the hematopoietic effects of NK-1 depend on the interacting ligand. Specifically, SP mediates hematopoietic stimulation[26], while its fragment, $SP_{(1–4)}$, inhibits hematopoiesis[26]. The effects of $SP_{(1–4)}$ have physiological significance since the SP fragment could be the product of digestion of SP by endogenous endopeptidase (Fig. 4)[26]. $SP_{(1–4)}$ binds non-covalently to the smaller of two binding pockets within the extracellular domain of NK-1[26]. $SP_{(1–4)}$ competes for NK-1 with the parent peptide, SP. The interactions between NK-1 and $SP_{(1–4)}$ are functional, since this leads to the production of negative regulators of hematopoiesis, TGF-β and tumor necrosis factor α[26, 29]. It is deduced that $SP_{(1–4)}$ is a method by which the tachykinins exert their own negative feedback on hematopoiesis.

To summarize the hematopoietic effects of NK-1 and NK-2, NK-1 mediates dual effects, positive and negative, depending on the interacting ligand. As per the current reports, NK-2 has been shown to interact with NK-A to mediate inhibitory effects on hematopoiesis[23]. The hematopoietic effects mediated by NK-1 and NK-2 are regulated partly through crosstalk between each other. In the presence of SP, NK-1 is expected to mediate hematopoietic stimulation[23]. This stimulatory effect is balanced by NK-2[5]. If intracellular signaling of NK-2 is blocked, the stimulatory effect of SP/NK-1 interaction is significantly increased. The modulatory effects of NK-1 and NK-2 on each other are important for maintaining a balanced outcome on hematopoiesis. An understanding of the mechanisms by which NK-1 and NK-2 exhibit crosstalk is limited, and is thus a subject of intense research. To unravel the mechanisms by which NK-1

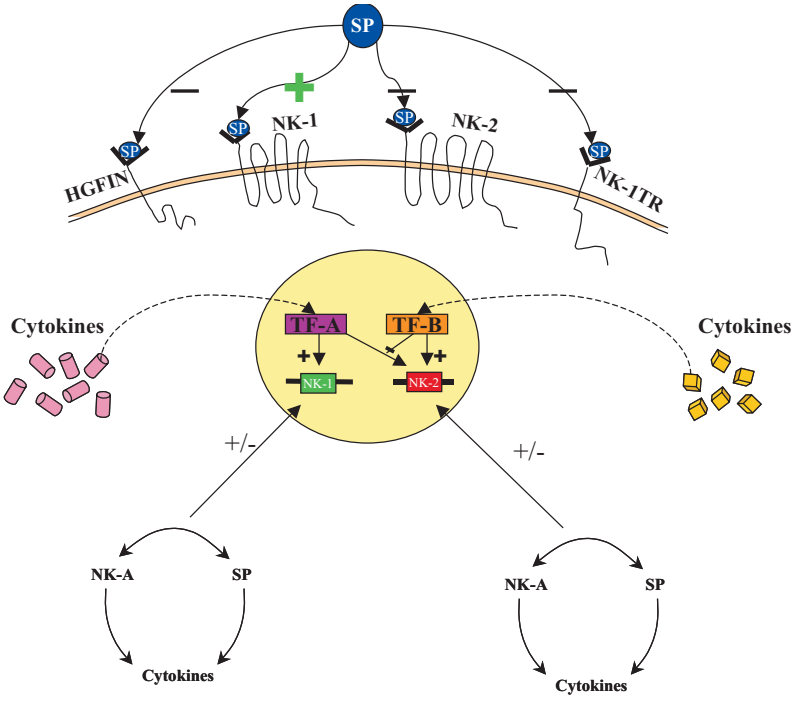

Figure 3. Mechanisms by which SP, NK-A, and cytokines are linked to NK-1 and NK-2 in hematopoietic cells. NK-1 mediates hematopoietic stimulation when it interacts with SP and negative hematopoiesis when it binds to NK-A[52, 61]. In BM stroma, both HGFIN and NK-1-truncated (NK-1-Tr) might act as decoy receptors by interacting with SP. The result of this is that SP is not longer available to NK-1. Cytokines produced as a result of SP-NK-1 or NK-A-NK-2 interactions led to the activation of particular transcription factors (e.g. TF-A or TF-B as putative factors). Depending on the transcription factor, either NK-1 or NK-2 would be induced. In summary, cytokines, SP and NK-A mediate positive or negative effects on the induction of NK-1 and NK-2.

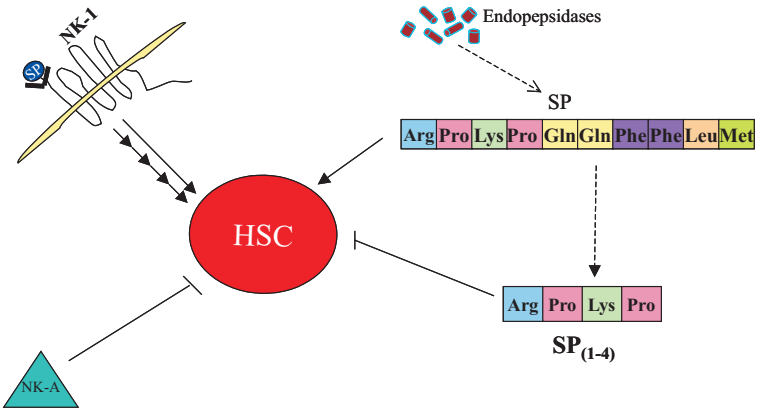

Figure 4. Regulatory effects of PPT-1 peptides on HSCs. SP/NK-1 interaction mediates hematopoietic stimulation through direct interaction (straight arrow) or indirectly through the production of cytokines (fragmented arrows). Endopeptidases use SP as a substrate to produce $SP_{(1–4)}$, which inhibits the proliferation of HSCs. The effects of $SP_{(1–4)}$ on HSCs parallel those of NK-A.

and NK-2 exhibit crosstalk will be important, since the tachykinins are linked to immune protection and stem-cell dysfunction[23].

Potential confounds on NK-1 by other molecules

Through molecular mimicry of parts of NK-1, SP interacts with HGFIN, also referred to as nmb (accession no. AAG42839), a single transmembrane protein located on chromosome 7[6, 71]. Parts of fibronectin also mimic the NK-1 binding site so as to allow non-covalent interactions between SP and fibronectin[59]. Computer analyses indicate species differences in the 5' flanking region of HGFIN from the homologue in mice and rats, osteoactivin[6, 41]. These differences might be sufficient to cause species-specific function of HGFIN. Regardless of these differences, a common function between HGFIN and osteoactivin is their similarity with respect to their involvement in cell differentiation[6, 64]. Evidence for HGFIN in cell differentiation is based on its expression in differentiated BM cells compared with undetectable levels in BM progenitors[6]. This suggests that HGFIN might be important to the cell-cycle checkpoint. In fact, consensus regions for multiple p53 sites are found in the 5' flanking region for HGFIN. Current research studies are in the process of identifying the role of HGFIN in hematopoiesis. Since NK--A inhibits cell proliferation of BM progenitors[61], studies aimed at addressing a link between NK-A and HGFIN are required to add the dissected pieces of tachykinins in hematopoiesis.

The similarity between regions of fibronectin and the ligand-interacting site of NK-1 is relevant to the emerging immune system in many respects. SP affects hematopoiesis at multiple levels, and should be protected from rapid degradation by endogenous endopeptidases[23]. Protection from degradation can occur strongly when SP is kept in close proximity to HSCs and progenitors. Through interactions with fibronectin, a component of extracellular matrix proteins, SP is protected from endogenous endopeptidases, and could be made available to the HSCs[60].

Tachykinins as a checkpoint in cell transit to the BM

In general, blood and nerve fibers follow similar paths to the BM. Examination of the literature describes nerve endings in close contact with reticular cells[73]. Since MSC are the cells referred to as reticular cells, anatomical literature suggests that nerve endings might form synapse-like structures with MSC. The question is why MSC surround blood vessels within the BM and what the possible physiological roles of the innervated fibers being in close contact with MSC are[11]. Answers to these questions could be extrapolated by analyzing the expression of NK-1 and NK-2 on MSC. RT-PCR studies show that the yin-yang type of expression between NK-1 and NK-2 in BM stroma is different from MSCs[23]. Both NK-1 and NK-2 are coexpressed in MSC, similar to reports for neural cells[23, 42, 74]. Although it might be premature to think that there is a parallel between MSC and neural cells based solely on the pattern of NK-R expression, it appears that coexpression of NK-Rs might allow for the MSC to respond rapidly to neurotransmitters derived from nerve fibers close to MSC.

To fully understand the role of NK-1 and NK-2 on MSC, a hypothesis can be formulated based on other related information on hematopoiesis and the biology of MSC. Although MSC express major histocompatibility complex (MHC) class II, currently there is no evidence of antigen-presenting abilities by MSC. MSC have been reported to exhibit immunosuppressive properties. Thus we hypothesize that the role of MHC class II on MSC is to be able to provide information to exiting immune cells that the barrier between the periphery and the BM is self. Perhaps the immunosuppressive effects of MSC might be

responsible for preventing an exacerbated immune response when there is a challenge from invading pathogens. This type of protecting might be considered as a "gatekeeper" function, in which the MSC regulate the movement of cells in and out of the BM. A relevant scenario would be pre-B cells or mature B cells exiting the BM. If the immunoglobulin gene does not undergo proper editing or if the B cell cannot recognize the MHC as self, the MSC could perhaps facilitate the elimination of auto-reactive immune cells prior to their circulation in the periphery. These exciting hypotheses need to be tested, since the answers will unravel an area of immune-cell development that could be considered the period where newly developed immune cells leave the BM to the peripheral lymphoid system.

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