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TACHYKININS IN THE EMERGING IMMUNE SYSTEM: RELEVANCE TO BONE MARROW HOMEOSTASIS AND MAINTENANCE OF HEMATOPOIETIC STEM CELLS

Steven J. Greco [1], Kelly E. Corcoran [1], Kyung Jin Cho [2] and Pranela Rameshwar [3]

[1] Graduate School of Biomedical Sciences, Interdisciplinary Program, UMDNJ, Newark NJ, [2] Department of Clinical Laboratory Science, College of Health Sciences, Korea University, Seoul, Korea, [3] Department of Medicine-Hematology/Oncology, UMDNJ-New Jersey Medical School, Newark NJ

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1. ABSTRACT

The mammalian tachykinins mediate crosstalk within the neural-immune-hematopoietic axis. Hematopoiesis occurs in the adult bone marrow (BM) and it is described as the method by which the immune system is replenished by a finite number of hematopoietic stem cells (HSC). These cells are found in the BM close to the endosteum where the oxygen level is the lowest. The BM is also resident to mesenchymal stem cell (MSC). The functions of HSC depend on the MSC to generate the supporting stromal cells. This review discusses possible mechanisms by which the MSC act as the `gatekeeper' in the BM and regulate immune cells in and out of the BM. The roles of the tachykinins are discussed in the context of homeostasis in the BM and as mediators of BM disruption. The involvement of the tachykinins within the BM microenvironment and the development of immune cells in the BM are explained.

2. INTRODUCTION: HEMATOPOIESIS: AN OVERVIEW

The adult bone marrow (BM) is the major site of hematopoiesis. The process of hematopoiesis is explained by the development of immune and other blood cells from a relatively small number of self-renewing hematopoietic stem cell, HSC (1, 2). The HSC are mostly found in areas close to the endosteum of the BM cavity where stromal cells are also located (figure 1). BM stromal cells support the functions of HSC and are involved in maintaining homeostasis in the BM (3). Thus, the adult BM is considered the primary organ of the emerging immune system.

Figure 2 shows a cartoon of the hierarchy within the hematopoietic lineage. A HSC can commit towards a myeloid or lymphoid progenitor that is generally referred to as the common myeloid (CMP) or common lymphoid (CLP) progenitors (4). CMP could be further subdivided in other lineages to generate non-lymphoid cells such as dendritic cells, erythrocytes, megakaryocytes and granulocytes (1, 4, 5). CLP generate progenitors that mature into T-, B-, natural killer and dendritic cells (5, 6). T-lymphopoiesis begins in the BM and is finally differentiated into T-cells in the thymus (7).

Hematopoiesis is regulated by different growth regulators such as cytokines, chemokines, neurotrophic factors, neuropeptides, neurotransmitters and extracellular matrix proteins (8). There is no direct association between a particular family of hematopoietic regulators and a function, since the hematopoietic function of a growth factor is not `mutually exclusive' of other mediators within the BM microenvironment (8, 9). Rather, different types of hematopoietic growth factors form a complex/interactive network that leads to the ordered biological functions in the BM. Although hematopoietic regulators are considered to be functionally redundant, they nonetheless exert specific functions in a particular microenvironment.

Hematopoiesis also involves cell-cell interactions, which are partly attributed to adhesion molecules (9-11). BM stromal cells support the self-renewal

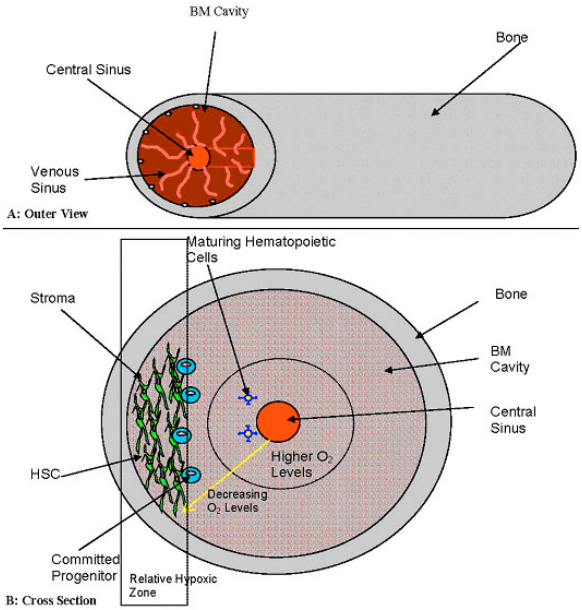

Figure 1. Cartoon of the structure of the BM cavity. A. Outer view of bone displaying central sinus and connecting vessels; B. Cross-section of BM: Gradient changes of O_2 from the sinus to the trabecula are shown. HSC-Stromal interactions are depicted close to the endosteal region (9, 85). The mature immune cells depict movement from an area where development was initiated (close to the endosteum) towards the sinus where the cell could enter the lymphatics.

and maturation of HSC, and also protect HSC from potential insults by chemical exposures, viral infection and anoxia (9). The cartoon, shown in figure 3 depicts the spatial and functional relationships between HSC and BM stroma.

3. NEUROENDOCRINE-IMMUNE-HEMATOPOIETIC AXIS

Although cytokines are generally considered as the prototype hematopoietic regulators (8), other molecules are involved in hematopoiesis. Neuropeptides, neurotransmitters, neurotrophic factors and neurohormones are major categories of hematopoietic regulators (12-18). The effects of neurotransmitters on hematopoiesis are in the `infant' stages of research. More in-depth research in this area will provide a better insight into the neuroendocrine-immune-hematopoietic axis. The anatomical substrates underlying the neural-hematopoietic axis involve neurotransmitters released from the sympathetic and peptidergic nerve fibers in the BM (19-24).

The BM is innervated by sympathetic and peptidergic nerves (25). Tachykinin positive nerve fibers are reported within the BM and in areas within the trabecula (26-29). Innervation to the BM is not unique since similar relationships exist for the thymus and secondary lymphoid organs (28). *In vitro* studies on the effects of neurotransmitters on hematopoiesis are based on the presence of the respective nerve fibers and receptor expressing cells in the BM (30-39).

Other studies also support a neuroendocrine-hematopoietic link. Stimulation of the sympathetic trunks in rats led to changes in BM cellularity and organ distribution (40-42). Experimentally induced epileptic seizures led to altered hematopoietic functions (43). In humans, hematopoiesis is suppressed in areas of the bone below spinal cord injury (44). Surgical trauma also causes BM disruption, with the loss of primitive and mature BM progenitors to the peripheral circulation (45). BM innervation allows for anterograde and retrograde communication within the neural-hematopoietic axis (46-52). The retrograde arm transports soluble factors and ligand-receptor complexes from the BM to neural bodies (52). The anterograde arm could release neurotransmitters from the autonomic postganglionic endings to the primary and secondary lymphoid organs.

3.1. Tachykinins

The neuropeptides, substance P (SP) and neurokinin-A (NK-A) belong to the tachykinin family of peptides (53). SP and NK-A are 11- and 10- amino acid peptides respectively and represent major peptides produced from the preprotachykinin-I gene (*PPT-I*) (53). The PPT-I gene undergoes alternate splicing and post-translational modification to form four transcripts: alpha-, beta-, gamma- and delta-*PPT-I* (figure 4). Exons 3 and 6 encode SP and NK-A respectively. The fact that Exon 3 is present in each transcript indicates that SP could be the most likely peptide produced from the PPT-I gene (54).

Another tachykinin peptide, hemokinin 1, has been identified in hematopoietic cells (55). Hemokinin 1 is derived from the PPT-C gene (55). Viral proteins have also been reported to mimic the tachykinins. Virokinin, released from viral-infected cells, shows homology to the tachykinins with respect to immunoreactivity, and also interacts with the NK-1 receptor (56).

The tachykinins are released in the BM as neurotransmitters and are produced endogenously from BM-resident cells (37, 38). The tachykinins interact with specific receptors on BM-resident cells to regulate hematopoietic functions (37, 38). As hematopoiesis is the method by which the immune system is replenished, the tachykinins could be considered as key components in linking the neural-immune-hematopoietic axis.

3.2. Neurokinin (nk) receptors

The tachykinins bind with different affinities to three cloned neurokinin (NK) receptors: NK-1, NK-2 and NK-3. The NK receptors belong to a large superfamily of integral membrane G-protein-coupled-receptors (GPCR) with seven transmembrane loops. SP and NK-A show binding preferences for NK-1 and NK-2 respectively (57). NK-B, derived from the PPT-B gene, shows binding preference for NK-3 (58). NK receptors are ubiquitously expressed, e.g., T- and B- cells, monocytes/macrophages, hematopoietic progenitors, endothelial cells and BM stroma (57-60). Despite the widespread tissue expression of NK

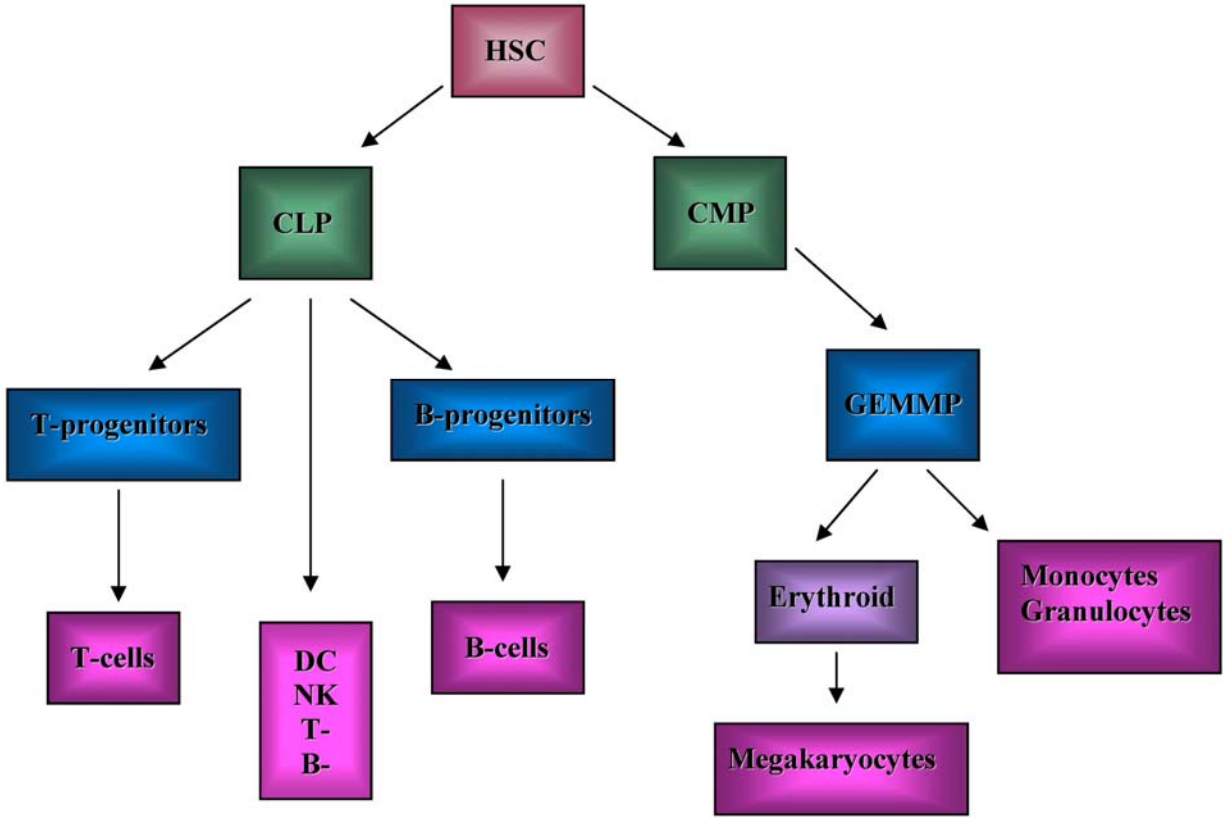

Figure 2. Hierarchy of hematopoietic cells. HSC are subdivided into two major lineages (1, 2). NK: Natural Killer Cells; DC: Dendritic cells; GEMMP: Granulocyte-Erythroid-Monocyte-Megakaryocyte progenitor.

receptors, the specific NK receptor subtype and its molecular regulation is tissue-specific. An example of specificity can be demonstrated by the differences in NK-1 expression in BM stroma and neural cells (61). The distribution of NK-3 on BM resident cells is unclear. As far as we are aware, there is no report of NK-3 expression on primary BM cells, however, NK-3 has been detected on a stromal cell line (30). In contrast to NK-3 expression, there is no question regarding the expressions of NK-1 and NK-2 on BM cells (62). NK-1 is activated and desensitized by ligand-dependent mechanisms (63).

A truncated form of NK-1 (NK-1-Tr) with 100 residues omitted in the carboxyl/cytoplasmic terminus is reported in human cells (64-65). NK-1-Tr is thought to form by alternative splicing of the precursor NK-1 mRNA. Compared to the full-length NK-1, the truncated form is less prone to desensitization and internalization (57). The experimental evidence suggests that resistance to desensitization could be explained by the loss of key phosphorylation substrates within the omitted part in the carboxyl region (57).

We are currently examining the physiological role of NK-1-Tr in the BM. Our approach is to understand the role of NK-1-Tr in malignancies and then extend the findings to BM functions. We have implicated NK-1-Tr in the development of breast cancer (Unpublished). Since breast cancer patients succumb to BM metastasis, the role of NK-1-Tr as a mediator of hematopoietic disruption by cancer cells is yet to be determined.

At this time, further research is needed to understand NK receptors in hematopoiesis. However, we have extrapolated related biological information to speculate on the roles of NK receptors on hematopoiesis. It is tempting to suggest that the differences in NK receptor expressions in BM and neural cells might be linked to developmental processes during embryogenesis. This premise is based on the different germ layers from which neural and BM stroma/HSC originate (66). While NK-1 is inducible in BM stromal cells, in neural cells, its expression is constitutive (37). This difference is partly explained molecularly by a specific region within the 5′ flanking region of the NK-1 gene (61).

NK-1 is induced in BM stroma by cytokines that show properties as hematopoietic stimulators (38). Furthermore, the genes for NK-1 and PPT-I are mostly induced by common cytokines (62). This common link between NK-1 and PPT-I is consistent with their roles as mediators of hematopoietic stimulation (37). NK-2 is constitutively expressed in unstimulated stroma unless NK-1 is induced (38). Although it is unclear how the yin-yang between NK-1 and NK-2 occurs, receptor crosstalk appears to be partly responsible (67). In summary, similar to the PPT-I gene, the genes for NK receptors could be argued as potential links within the neural-immune-hematopoietic axis.

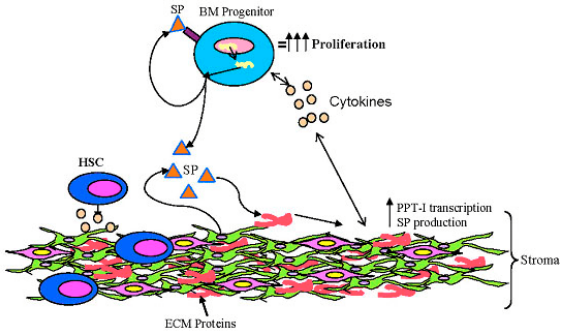

Figure 3. Functional and spatial dynamics of SP with components of the BM. SP mediates the proliferation of BM progenitors (shown as triple arrows). Cytokines stimulate both BM progenitors and stromal cells and are also produced by both cell subsets. Stromal cells could respond to cytokine production by expressing the PPT-I gene. SP can be mobilized by extracellular matrix proteins (ECM). HSC are shown within the location of BM stromal cells (Reviewed in reference 62).

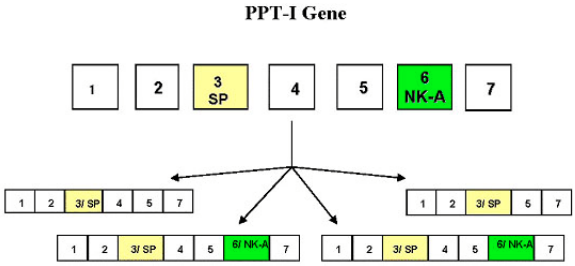

Figure 4. Processing of the PPT-I gene. The four derived transcripts give rise to multiple peptides with SP and NK-A as the major peptide. The four transcripts in counterclockwise order starting from the top left transcript are: alpha, beta, delta and gamma transcript.

3.3. Tachykinins in hematopoiesis

The roles of PPT-I-derived tachykinins have been mostly studied in the erytho-myeloid compartment (30, 62, 68, 69). In the lymphoid compartment, SP functions as a terminal differentiation co-factor for B-cell maturation (70). In the erythro-myeloid lineages, SP mediates stimulatory effects on granulocytic-monocytic and erythroid progenitors (37). Table 1 shows a summary of previous findings on PPT-I peptides and hematopoietic effects. Unpublished studies in our laboratory show that SP mediates an increase in the proliferation of megakaryocytes. We are unaware of any report that shows a role for the tachykinins on T-lymphopoiesis in the BM. However, SP has a role in T-cell development after the T-cell progenitors have migrated from the BM to the thymus (71). Hemokinin-1 is involved in the regulation of lymphopoiesis in both the B- and T- cell compartments (72).

The hematopoietic effects of SP are partly mediated through the production of cytokines in BM resident cells (table 1). BM stromal cells are the major sources of tachykinin-induced production of growth factors (62). The broad-acting cytokine, IL-1, induces the production of positive and negative hematopoietic regulators and also mediates indirect hematopoietic effects through the production of other growth factors, such as stem cell factor (73, 74). The specific cytokine induced by SP or NK-A correlates with the hematopoietic effect of the tachykinin (37, 38). BM-derived factors that have been shown to have roles in SP-mediated hematopoietic effects include IL-1, IL-3, GM-CSF, and SCF (37).

In contrast to the stimulatory hematopoietic effects of SP, the effects of NK-A are complex. NK-A, through NK-2, mediates inhibition on the proliferation of granulocytic-monocytic progenitors and exhibits mixed effects on erythroid progenitors (table 1 and figure 2). NK-A has been shown to stimulate early (BFU-E) and late (CFU-E) erythroid colonies (table 1). The hematopoietic effects of NK-A are mostly indirect, through the production of cytokines in BM stroma: macrophage inflammatory protein-1alpha (MIP-1alpha) and transforming growth factor-beta (TGF-beta) (37).

Similar to NK-1, NK-2 activates phospholipase C-linked pathways (58). The roles of these second messengers in the crosstalk between NK-1 and NK-2 are yet to be determined. The role of NK-2 in hematopoiesis depends on NK-1 being downregulated (67). The opposite effect is observed for NK-1 (67). Thus, `fine-tuned' regulation of NK-1 and NK-2 at the molecular levels will begin to dissect how these two receptors can be involved in maintaining tissue homeostasis in the BM.

Despite homology in the primary structures of the tachykinins, each mediates a distinct hematopoietic function (37, 38). SP, the major peptide of the *PPT-I* gene, mediates the proliferation of the mature and immature hematopoietic progenitors (37). In contrast, NK-A inhibits hematopoiesis (37). Elucidation of the complex biological functions of SP is confounded by the presence of endogenous endopeptidases. These endopeptidases can use PPT-I peptides as substrates to produce smaller peptides with biological activities. An example is the amino fragment of SP, termed SP (1-4), which mediates hematopoietic suppression through NK-1 (75). It should be noted that although SP and its fragment share the NK-1 receptor (75), each mediates opposing hematopoietic functions. The NK-1 receptor is developmentally regulated in BM-derived mast cells (60). Similar development might not be observed in hematopoietic cells of other lineages since NK-1 expression is not detectable on unstimulated immune cells (76).

3.4. Negative feedback of the tachykinins on hematopoiesis

Multiple mechanisms appear to be operative with regards to negative regulation on hematopoiesis by the tachykinins. SP could act as its own feedback through desensitization of the NK-1 receptor following prolonged exposure to the ligand (57). NK-A mostly exerts inhibitory effects on hematopoiesis (table 1). Cytokines (TGF-beta and MIP-1alpha) produced by NK-A are not only important in hematopoietic inhibition, but could also turn off the transcription of the NK-1 gene (37, 38). An understanding of SP is difficult since this cytokine induces the production

Table 1. Effects of SP and NK-A on hematopoiesis

	SP induces **IL-1, IL-2, IL-3, c-kit, IL-6, TNF-α, IFN-γ, G-CSF**	**NK-A induces** **MIP-1α, TGF-β**
Effects on CFU-GM	SP: Stimulatory	NK-A: Inhibitory
Effects on CFU-E	Stimulatory	Stimulatory
Receptors	SP mediates via NK-1	NK-A mediates via NK-2
Effects on primitive BM progenitors	Stimulatory	No effect

The summary in Table 1 (37, 38) shows mostly hematopoietic stimulatory cytokines induced by SP and inhibitory-linked cytokines by NK-A

of multiple cytokines that have properties at different levels of the hierarchy of the HSC and its branching lineages (figure 2). Kresberg *et al.* reported that the hematopoietic outcome of SP depends on opposing effects of cytokines produced in the reaction (77). This supports a major influence of the BM microenvironment in tachykinin-mediated BM functions.

Mediators other than cytokines could be involved in the negative effects of NK-A on hematopoiesis. We propose that cell cycle regulators, such as p53, could interact with the NK-2 promoters to regulate its expression in BM cells (67). Consequently, NK-2 induction would lead to the downregulation of NK-1 and cell cycle arrest of HSC. Together, the argument for NK-A-NK-2 interactions would be protection of the finite number of HSC. SP (1-4), the amino terminal fragment of SP, could negatively regulate hematopoiesis by blunting the proliferation of the more primitive progenitors and inducing the release of TGF-beta (75). This negative feedback by SP (1-4) relies on the presence of endogenous endopeptidases (75). Thus, if the activities of endopeptidases are disrupted, SP would be protected and its accumulation could lead to overactivation of hematopoiesis, with the threat of multiple genetic mutations in HSC.

3.5. BM stroma in tachykinin-mediated effects on hematopoiesis

BM stromal cells are differentiated from mesenchymal stem cells (MSC) (78). Stromal cells are located close to the endosteal region of the BM with the lowest level of oxygen (figure 1). HSC are also located within the same region of the BM. This allows interactions between HSC and BM stroma (figure 1). Hematopoietic effects by SP and NK-A are mostly indirect, through BM stroma (37, 38). SP and NK-A induce the production of cytokines in BM stromal cells (32, 37). Thus, the stromal cells can be considered as a key to the development of an intricate network that involves cytokines, SP, NK-A and other molecules. Such a network is important because the daily need of immune cell and blood replacements for a healthy individual is dynamic with an average of 10^{12} cells/day.

During acute need for hematopoietic activity, as required in surgical trauma of an individual who was otherwise healthy prior to the trauma, the BM stroma appears to be the limiting compartment for this type of acute hematopoietic responses (45). Studies with surgical trauma patients and spinal cord injury suggest that the BM is unable to retain immature BM progenitor cells (44, 45). In animals, denervation of nerve entering the BM caused rapid loss of BM cellular compartment (35).

Two examples to show major roles of BM stroma in PPT-I-mediated effects on hematopoiesis are discussed: I. During hemorrhagic shock, BM stroma might respond by expressing the PPT-I gene so as to induce the production of hematopoietic stimulatory cytokines (79). II. During acute infection, stromal cells could respond to the invading organism by expression of the PPT-I gene. This would lead to the production of additional immune cells needed to protect invading organisms. Such assumptions are made because macrophages within the BM can express the genes for PPT-I and NK receptors following immune challenges (80).

3.6. Confounds on hematopoiesis by molecules that mimic NK-1

Earlier studies suggested that the NK receptors that were expressed in neural and BM cells might be different. Subsequent identification of at least two molecules and the cloning of NK-1-Tr in BM cells indicate that the original premise of NK subtypes might be explained by molecules that mimic NK-1 (81, 82). Two molecules are worth discussing since they have been shown to interact with SP and are relevant to hematopoiesis: Fibronectin and the HGFIN (81, 82).

Fibronectin is a component protein of the BM microenvironment extracellular matrix (81). Extracellular matrix proteins are indispensable to the functions and protection of the limited HSC (figure 3). HGFIN is a single transmembrane protein that has been shown to be expressed on BM stroma and immune cells (82). Since immune cells are the matured cells of hematopoiesis, experimental evidence suggests that HGFIN might be relevant to terminal differentiation of hematopoietic progenitors (82). In addition to the differentiation step, HGFIN might also be important to the functions of HSC (figure 2), as suggested by its induction in BM stroma (82). In summary, it could be argued that HGFIN is important to at least two levels of the hematopoietic hierarchy: I. The upper level of the hematopoietic hierarchy (figure 2) when the stromal cells interact with the HSC and, II. The lower end of the hierarchy where differentiated immune cells are generated to replenish the immune system. Current research studies are aimed to unravel the molecular interactions among SP, HGFIN and NK-1. Despite homology between NK-1 and NK-2, we have not found possible similarities between NK-2 and HGFIN or fibronectin.

The significance of SP-fibronectin interactions is relevant to homeostasis because fibronectin would be able to mobilize SP so as to make this tachykinin available to

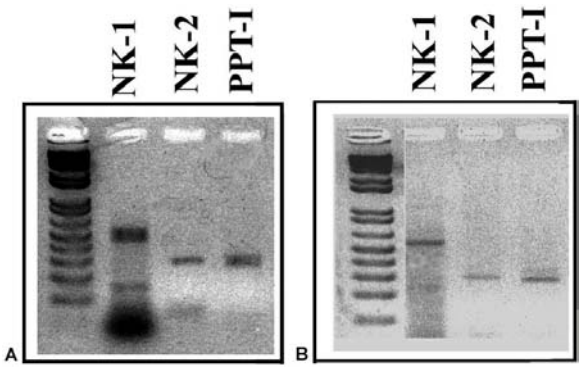

Figure 5. Expression of PPT-I, NK-1 and NK-2 in SP- or IL-1alpha-stimulated MSC. Total RNA was extracted form MSC that were stimulated with 10 nM SP (**A**) or 10 ng/ml IL-1alpha (**B**) for 12 h. Primers specific for PPT-I, NK-1 or NK-2 were used in RT-PCR. Figures shown are representative of five different experiments for PPT-I (315 bp), NK-1 (650 bp) and NK-2 (279 bp). Primers and the methods were previously described (67, 75).

the nearby HSC (figure 3). Indeed, experimental studies show that fibronectin-SP interactions are relevant to normal homeostasis since fibronectin protects SP from degradation by endogenous endopeptidases (83). This finding was important because if SP is degraded by endopeptidases, the final product is a small peptide, SP (1-4), that could negatively affect the hematopoietic effects of SP (75). The protective property of fibronectin for SP could also lead to BM dysfunction.

Leukemias are sometimes associated with the development of BM fibrosis (62). Increased production of SP has been reported in several types of leukemias (62, 84). These patients also have increased levels of fibronectin in the BM and in their circulation (81). This increase in fibronectin could protect SP from degradation and, ultimately, lead to its accumulation. SP mediates properties that are amendable to BM fibrosis, such as: stimulation of angiogenesis, and being mitogenic to fibroblasts/stroma (39, 62). At this time, a cause-effect relationship between SP-fibronectin and BM fibrosis has not been demonstrated. However, correlation studies strongly suggest the elevated complexes of fibronectin-SP in the circulation and BM of patients might be important in the pathophysiology of the disease.

A recently identified peptide, vitronectin, is a virus-derived protein that shows similarity to the carboxyl part of the tachykinins (56). Vitronectin can also interact with the NK-1 receptor (56). This finding is significant to BM biology because many HSC disorders are virally mediated. This report by Zimmer *et al.*, (56) indicates that hematologists need to examine the literature on stem cell disorders and verify that BM dysregulation is not from a tachykinin-related peptide, such as vitronectin, or perhaps other unidentified homologues of tachykinins.

3.7. Mesenchymal stem cells (MSC)

Hematopoiesis is inclusive of another BM-resident stem cell, MSC (78). These alternate stem cells could provide clues to tachykinin-mediated effects on hematopoiesis. Evidence for this will be discussed in this section. In contrast to the blood forming potential of HSC, MSC differentiate into cells such as stromal cells (78). In this regard, MSC and HSC can be considered as cells that are functionally connected, since the stromal cells are required to maintain the properties of HSC (figures 1 and 3). Anatomically, the locations of MSC and HSC in the BM are separate: LHSC prefer the area with the lowest oxygen levels, which is close to the endosteal region or in the periphery of the bone (85). MSCs surround the vasculature system of the BM (78). This location of MSC allows for contact with nerve fibers, based on the common paths of blood vessels and nerve fibers into the BM. The location of MSC around BM vessels indicates that cells out of the BM must bypass MSC before they transit the endothelial cells of the blood vessels to the periphery/lymphatic system.

Morphologically, MSC are reticular-type cells (86). Anatomic and electron microscopic studies suggest that nerve fibers form synapse-like structures with MSC (reticular-type cells) (86). This is consistent with nerve fibers and blood vessels following along the same paths (discussed above). Recent studies support the close location of BM nerve fibers with MSC (39). The possible synapse-like structures with MSC and nerve fibers in the BM (86), combined with the presence of receptors for neurotransmitters on MSC (figure 5) indicate that functions of MSC are at least partly regulated by neurotransmitters. Although BM stromal cells are progenies of MSC (86), the pattern of neurotransmitter receptors is different between MSC and its daughter stroma (figure 5). For example, in stroma, NK-1 and NK-2 are not coexpressed (38) whereas in MSC, both receptors are coexpressed (figure 5).

MSCs are evolving as the major stem cells in tissue repair medicine (87).While it is still unclear whether HSC transdifferentiate, MSC are accepted as an adult stem cell with the potential to differentiate and transdifferentiate into cells of various tissues. The allogeneic effects of any stem cell preclude ease for application in repair medicine in an unrelated host. MSC have unique immune properties that would allow them to bypass rejection in an allogeneic host (88). Veto properties of MSC indicate that these stem cells can suppress an allogeneic response when they are placed as third party cells in an ongoing immune reaction (88). The veto properties of MSC are specific, since MSCs do not affect responses to specific recall antigens. We propose that the multiple immune properties of MSC are considered in the context of their location around the BM vasculature system. One could surmise that MSC might be the gatekeeper cells of the BM and perhaps explain the low frequencies of stem cell disorders in individuals below 40 years. Recent studies show that PPT-I deficient BM cells confer protection from neurogenic inflammation of the lung (88). These observations suggest that PPT-I might be important to cells that home to the lung. Although the authors (88) did not identify the candidate cells, it would be interesting to determine whether the `plastic' nature of MSC make them candidates for protecting the lung.

3.8. Tachykinins in hematopoietic stem cell disorders

BM fibrosis is common to several hematological disorders, in particular, myeloproliferative disorders, MPD

(62). BM fibrosis is characterized by fibrosis, hypercellularity and excessive deposits of extracellular matrix proteins. The disorder also displays dysregulated production of fibrogenic, proinflammatory and angiogenic cytokines (62). Chronic fibrosis leads to BM dysfunction and extramedullary hematopoiesis (62). The heterogeneity of BM fibrosis leaves few options for therapeutic intervention. Hematopoietic stem cell replacement could favor the young, however BM fibrosis mostly occurs in the aged. Future studies to understand the biology of BM fibrosis will provide therapeutic interventions.

SP levels are increased in the circulation of patients with BM fibrosis and detectable in their BM (62, 81). The increased SP are found in complexes with fibronectin in the BM and periphery of patients with BM fibrosis (62, 81). In healthy BM, fibronectin mobilizes and protects SP. However, fibronectin-SP levels in healthy individuals are below detection by currently used techniques (83). Perhaps the high levels of SP-fibronectin might explain the activation of monocytes and dysfunction of megakaryocytes in patients with BM fibrosis. The source of SP in patients with BM fibrosis is unclear. Monocytes and macrophages cannot be eliminated since these cells are activated in the patients, and are also capable of expressing the PPT-I gene (62, 80). Activated monocytes from patients with BM fibrosis produce high levels of IL-1 (62), which could induce the expression of the PPT-I gene by autostimulation.

The pathophysiology of BM fibrosis is consistent with the functions of SP, e.g., increased angiogenesis in the BM of patients (62), increased proliferation of BM fibroblasts (62), increased production of fibrogenic cytokines (62, 90) and enhanced production of cytokines, known to be induced by SP. As BM fibrosis develops secondarily to the underlying clonal disorder of the HSC, the question arises as to the time at which SP appears. SP has been reported (62, 84) to be produced in clonal HSC. This suggests that in some cases, SP from the clonal stem cells might be the cause of BM fibrosis.

The PPT-I gene could be expressed during hypoxic conditions in the BM (79). This is relevant since the $p0_2$ level of BM aspirate is significantly lower than peripheral circulation (91). Theoretical models suggest that the BM has a gradient change of oxygen and that HSC are located in the region with the lowest oxygen, whereas committed progenitors are in relatively oxygenated areas (85). Since the PPT-I gene is induced by hypoxia (79), it would be interesting to study if lowered oxygen levels in the elderly might initiate dysregulated expressions of the PPT-I gene. As the PPT-I gene is already linked to leukemia (62, 90), a relative hypoxic state in the BM of the aged might be a predisposing factor for MPD.

3.9. Tachykinins in bm disruption

As described above for MDP, the homeostasis of tachykinins functions could be altered. BM disruption by the genes for PPT-1 and NK receptors could occur by cancer cells that invade the BM or by cancers that are developed from BM-derived cells. The latter includes leukemia and lymphoma (62, 90). The other category includes solid tumors, namely breast cancer, that shows preference for the BM (92). Endocrine- and neuroendocrine- related cancers show preference for the BM. Several of these cancers have been reported to constitutively express the PPT-I and NK receptor genes (62). This review focuses on breast cancer cells, since the studies show that the PPT-I gene mediates early entry and integration of breast cancer cells in the BM (92). Although early metastasis of breast cancer cells to the BM might not cause hematopoietic dysfunction, these cells could later invade the bone and cause BM disruption. Consequently, there would be destruction of the emerging immune system. The role of PPT-I in breast cancer relapse and bone invasion is yet to be determined.

3.10. Possible role for sp during HSC mobilization

CD26, also referred as DPPIV/dipeptidylpeptidaste IV can use several substrates. The substrates relevant to this review are SP and the chemokine, SDF-1alpha (93). HSC are attracted to SDF-1alpha (93, 94). However, inactivation of SDF-1alpha by CD26 enhances the mobilization of HSC to the peripheral circulation (95). SP induces the expression of SDF-1alpha (unpublished observations). Thus, it is likely that downregulation of SDF-1alpha by CD26 might occur indirectly through degradation of SP by CD26. Since mobilization of HSC is now becoming the standard method for BM transplantation, the peripheral cells need to be in cell cycle quiescence while retaining long-term self-renewal abilities. Thus, it would be logical for SP to be absent so that cytokines that are stimulatory to the HSC pool would be downregulated. In this regard, the presence of NK-A and SP (1-4) would be advantages since these tachykinins would blunt the proliferation of the mobilized HSC. In summary, it would be undesirable for SP to be within the microenvironment of the BM since the cells have to retain transplantable capabilities during the mobilization phase from the donor.

4. PERSPECTIVE

The tachykinins, in particular SP, its fragments and NK-A, have profound effects at many levels of the adult emerging immune system. The role of the tachykinins in the adult emerging immune system has to be demarcated from the ontogeny of the hematopoietic system in the embryo. The basis for caution is the differences in PPT-I regulation in embryonic stem cells compared to its differentiated form (96). The role of the tachykinins in the developing hematopoietic compartments within the intra- and extra- embryonic compartments has not been studied. Thus, the compiled information in this review cannot be extended to embryonic development of the hematopoietic system, such as the effects on hemangioblasts (66).

Hematopoietic homeostasis is maintained through `fine-tuned' balance by a particular peptide exerting either a stimulatory or inhibitory effect on hematopoiesis. The effects of the tachykinins are inclusive of the BM microenvironment. This is clearly demonstrated by the opposing functions of SP and its fragment, SP (1-4), as

hematopoietic stimulator and suppressor respectively. The effect of SP (1-4) occurs through the influence of the BM microenvironment. Endogenous induction of endopeptidases correlates with the long-term exposure of HSC to hematopoietic stimulators. By digesting SP to SP (1-4), the net effect is suppression of hematopoiesis and, ultimately, negative feedback.

The roles of the tachykinins are not limited to positive and negative effects of SP and SP (1-4) respectively. Computational studies confirm functional studies to show distinct binding pockets for SP and SP (1-4) (75). However, the molecular and functional relationship between SP and NK-1 are more complex, since recent reports show two binding pockets in NK-1 for SP (97). It is yet to be determined how SP discriminates one interacting site over another and/or if the functions mediated by a particular site are unique. Perhaps studies towards these questions will bring another level of understanding on tachykinins and hematopoiesis.

Studies with the tachykinins on malignancies in the BM are clear examples of methods by which the tachykinins can manipulate the BM microenvironment. Experimental models show that PPT-I peptides produced in breast cancer cells change the functions of BM stromal cells to allow for the quiescence of the cancer cells in the BM (92). Another example is shown in hematological disorders in which SP, produced by leukemia cells, led to disruption of the BM microenvironment. Dysfunctional processes are caused by overexpression of molecules, e.g., cytokines and extracellular matrix proteins. Ultimately, there is loss of BM homeostasis, partly through the development of BM fibrosis (62).

This review focuses on the tachykinins and BM functions with summaries that underscore the relevance of the tachykinins on hematopoietic homeostasis. Central to BM functions are the HSC, which are the prototypical adult stem cell and the most studied among somatic stem cells (98). Studies on other somatic stem cells parallel the information on HSC. Thus, the studies described in this review could be extrapolated to other stem cells in the neural and also non-neural systems. The identification of another tachykinin, hemokinin, and its ability to interact with both NK-1 and NK-2 (99) indicates that each of the tachykinins will have to be studied separately and in combination with other members of the tachykinin family. Continued research on the tachykinins in the emerging immune system is required, so as to identify novel mechanisms in hematopoiesis. Ultimately, the goal is to identify new targets for drug development to treat tachykinin-mediated diseases of the emerging and mature immune systems.

The tachykinins and their receptors are emerging as targets for clinical disorders (100). The redundancy of the emerging immune system by multiple tachykinins and by other related molecules could hinder or enhance such therapies. Hindrance could be derived by blunting effects during therapies by related molecules. Positive outcomes could occur by the redundant tachykinins being able to protect HSC from toxic effects. Regardless, these are issues that will have to be considered, as treatments to target the tachykinins for different disorders are developed.

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Send correspondence to: Pranela Rameshwar, Ph.D., UMDNJ-New Jersey Medical School, 185 South Orange Ave, MSB, Room E-579, Newark, NJ 07103, Tel: 973-972-0625, Fax: 973-972-8854, E-mail: rameshwa@umdnj.edu