REVIEW

The significance of substance P in physiological and malignant haematopoiesis

Michal Nowicki, Danuta Ostalska-Nowicka, Beata Kondraciuk, Bogdan Miskowiak

J Clin Pathol 2007;**60**:749–755. doi: 10.1136/jcp.2006.041475

The role of substance P (SP) in physiological haematopoiesis is well established. However, it also seems to be important in the neoplastic transformation of bone marrow, leading to the development of acute leukaemia in children, and also metastases to bone marrow of solid tumours (particularly neuroblastoma and breast cancer) in early stages of these diseases. This review summarises the available data on SP involvement in both processes. In the future, SP antagonists may be used as anti-neoplastic drugs, for example by direct or indirect blocking of tumour cell proliferation through inhibition of growth factor production and interleukin-1b synthesis.

Studies on bone marrow (BM) innervation have a long history,[1] and even though the presence of nerves in BM has been sufficiently well documented, the significance of substances released by the nerves during haematopoiesis remains unclear. In general, nerve fibres are provided to bones from an appropriate branch of the spinal nerve supplying a given region of the body. They include both extraganglionic sympathetic fibres and afferent sensory fibres originating from spinal ganglia. The classical and still referred to studies of de Castro[2] showed that the nerves penetrate the BM cavity in common with the nutrient artery, split into branches and follow bone marrow arterioles, entwining them in the form of a dense network. A more complete illustration of BM innervation and relations between nerve endings and microelements of the BM environment was presented by Yamazaki and Allen.[3] As many as 61.5% of myelinated fibres were found to terminate between cells of arterial adventitia, 37.8% between haematopoietic cells and only 0.7% on cells forming BM sinuses. The significantly more numerous non-myelinated fibres most frequently terminate between adventitial cells (66.9%), on smooth muscle cells forming walls of BM blood vessels (4.9%), on cells forming BM sinuses (9.7%), and directly between BM cells (18.5%). This indicates that substances released from nerve endings may participate in formation of the haematopoietic microenvironment.

The most richly represented nerve endings in BM include those which release substance P (SP) and calcitonin-gene related peptide.[4] Others, which are less frequent in BM nerve endings, release neuropeptide Y and vasoactive intestinal peptide.[5]

See end of article for authors' affiliations

Correspondence to: Dr Michal Nowicki, Department of Histology and Embryology, Poznan University of Medical Sciences, ul. Swiecickiego 6, 60-781 Poznan, Poland; mnowicki@amp.edu.pl

Accepted 10 December 2006
Published Online First 15 December 2006

HAEMATOPOIESIS

There are at least two stem cells in adult BM: the haematopoietic stem cell (HSC) and the mesenchymal stem cell (MSC).[6 7] The major function of HSC, which is typical also for most of the studied stem cells, is continuous replacement of all cells which constitute the immune and blood systems. The process known as haematopoiesis involves multidirectional regulations among haematopoietic cells, BM stromal cells and nerve endings.[7] HSCs are found in the low-oxygen areas of BM, close to the endosteum.[8] MSCs are located in the inner region of blood vessels.[9] Despite the anatomical distance between HSCs and MSCs, these two stem cells are functionally interconnected since MSCs are the source of the supporting stromal cells.[10 11]

Haematopoiesis within the BM microenvironment depends on multidirectional stimuli.[12] Cellular responses cause production of soluble factors such as cytokines, chemokines, neurotrophic factors and neuropeptides.[13] Innervation of the BM with peptidergic, including SP, and sympathetic fibres[14–17] suggests that the functions, and possibly the properties of HSCs could be influenced by the neural system. However, homoeostasis in the haematopoietic system is attained by multiple intracellular pathways triggered by receptors and their respective ligands. One group is composed of neurokinin receptors (NK-Rs) and neurotransmitters belonging to the tachykinin family of peptides.[18 19]

TACHYKININS

Neuropeptides belonging to the tachykinin family are encoded by three genes, namely *TAC1*, *TAC3* and *TAC4* (known previously as PPT-A, PPT-B and PPT-C belonging to the preprotachykinin (PPT) gene family).[20] The tachykinins are mostly 10–12-amino acid peptides that present a common carboxyl terminus, Phe–X–Gly–Leu–Met–NH2, where X is either an aromatic or branched aliphatic residue.[21] *TAC1* encodes two of the most studied tachykinins, SP and neurokinin (NK)-A.[21] SP and NK-A are 11- and 10-amino acid peptides, respectively. PPT-A comprises seven exons, which can be alternately spliced and modified to form four transcripts: α, β, γ, and δ. SP is encoded by exon 3,

Abbreviations: ALL, acute lymphocytic leukaemia; BM, bone marrow; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte monocyte colony stimulating factor; HK, haemokinin; HSC, haematopoietic stem cell; IL, interleukin; MSC, mesenchymal stem cell; NK, neurokinin; NK-R, neurokinin receptor; PPT, preprotachykinin; SCF, stem cell factor; SP, substance P

present in each transcript, and NK-A is encoded by exon 6, present only in transcripts β and γ.[21] *TAC1* gene is expressed in neural (peripheral and central) and non-neural tissues, BM, and immune cells.[22 23] *TAC3* has seven exons, with exon 5 encoding nerokinin B (NK-B)[20]; it is expressed in the brain and in peripheral tissues.[21] *TAC4* gene produces haemokinin 1 (HK-1), expressed in haematopoietic cells.[17 24 25] It appears that HK-1 is distinctively expressed outside the neural system and has a prominent role in the regulation of lymphopoiesis.[25] It is encoded by exon 2, found in each transcript.[21] Finally, *C14TLK-1* gene gives origin to endokinins A and B, expressed in heart, liver and placenta.[26]

Traditionally, neurons have been identified as the major source of SP, NK-A and NK-B, but now it is well established that tachykinins and their receptors are expressed in the cardiovascular system, salivary gland, skin, muscles, respiratory system, digestive tract, genitourinary tracts, thyroid gland and immune system.[25–28] Because of such diffuse expression and regulation of disparate physiological functions, tachykinins can also be implicated in the pathogenesis of many diseases, including neoplasms.

TACHYKININ RECEPTOR SUBTYPES

The tachykinins interact with three natural tachykinin (neurokinin) receptors: NK-1R, NK-2R, and NK-3R.[13] They belong to the family of 7-transmembrane, G-protein coupled receptors.[20] SP and HK-1 exhibit binding preference for NK-1R, whereas NK-A show binding preferences for NK-2R.[21 29] The tachykinins can, however, interact with weak binding affinity to other NK-Rs.[9] NK-Rs are widely expressed in neural and non-neural systems. BM stroma, immune, and haematopoietic cells also express NK-Rs.[13 20]

SUBSTANCE P AND ITS SIGNIFICANCE IN PHYSIOLOGICAL HAEMATOPOIESIS

Substance P (H–Arg–Pro–Lys–Pro–Gln–Gln–Phe–Phe–Gly–Leu–Met–NH_2) shows wide distribution in the nervous system, in which it plays the role of a neuromediator.[30 31] Outside the central nervous system SP is antidromally released from sensory nerve endings of C type nerve fibres[32 33] in response to mechanical, chemical, and thermal insults as well as in response to factors released at sites of tissue injury.[33–37]

According to several authors, the presence of peptidergic nerve endings in the closest vicinity of immunocompetent cells in organs most exposed to contact with foreign antigens represents an anatomical exponent of functional links between the mentioned fibres and cells and, more generally, between nervous system and immune system.[38–40]

In humans, receptors for SP can be demonstrated on around 40% of peripheral blood lymphocytes.[36] Compared to mature lymphocytes, lymphoblasts carry around 3–4-fold higher amounts of receptors for SP.[41] Apart from lymphocytes, receptors for SP are also found on monocytes,[42] endothelial cells,[43] fibroblasts[44] and haematopoietic cells.[45] Substance P augments proliferative activity of human and mouse T lymphocytes,[46 47] human smooth muscle cells,[48] mouse fibroblasts,[49] fibroblasts of human skin,[44] smooth muscle fibres of arterial walls,[50] human synovial cells[51] and human cells forming colonies of granulocytes and monocytes or of erythrocytes.[52]

SP stimulates production of cytokines such as interleukin-1 (IL),[53 54] IL-2,[36 55] IL-3, IL-6, tumour necrosis factor-α,[51] interferon-γ,[56] granulocyte monocyte colony stimulating factor (GM-CSF) and stem cell factor (SCF).[55] It may intensify expression of adhesion molecules, i.e. intercellular adhesion molecule 1, which promote implantation of grafted haematopoietic cells.[57] Due to the presence in BM peptidergic nerve endings, SP has an easy access both to haematopoietic cells and to cells forming sublayers of BM.[58] Earlier studies showed that SP is also released in BM from macrophages,[59] eosinophils[60 61] and cells of vascular endothelium.[62] Most of cells present in BM, i.e. haematopoietic cells[52] and cells forming BM stroma,[44 63] as well as lymphocytes present there, particularly T lymphocytes,[64] are equipped with the SP-specific receptor, NK-1R. Tested in short term cultures of human BM in methylcellulose, SP alone was shown to support haematopoiesis in vitro.[52] The authors showed that SP, at a concentration of 10^{-11}–10^{-8} mol/l could substitute for IL-3, granulocyte colony stimulating factor (G-CSF) and GM-CSF, the presence of which was indispensable for growth of colonies. On the other hand, substance P could not substitute for erythropoietin although, when added together, it augmented activity of the latter. Specificity of this stimulatory action of SP was confirmed by administering it together with blockers of the known subtypes of SP receptor. Such a parallel administration of SP and a blocker for a subtype of NK-1R receptor yielded results at the control level. However, blocking of the NK-2R receptor had no effect on SP activity.[45] SP was also found to affect haematopoietic cells in an indirect manner, i.e. through the stromal cells, stimulating their production of cytokines. Supplementation of SP-stimulated cultures with antibodies specific for IL-1, IL-3, IL-6 and GM-CSF resulted in partial inhibition of cell growth, proving that SP can act through the induction of the cytokine synthesis. SP also induces synthesis of IL-1 and SCF in BM stromal cells.[65]

Cytokines linked to the haematopoietic functions of SP include IL-1, IL-3, GM-CSF and SCF.[23] SP induces production of these cytokines, which exhibit stimulatory effects on haematopoiesis. Alternatively, the cytokines induced by SP could activate BM cells through an autocrine and/or paracrine mechanism to produce other cytokines with haematopoiesis-stimulatory effects.[66] For example, SP induces the production of IL-1, which stimulates the induction of haematopoietic factors with direct and indirect effects on HSCs.[66] In contrast to SP, the haematopoietic effects of NK-A could be stimulatory or inhibitory, depending on the particular haematopoietic lineage.[52 67] NK-A inhibits the proliferation of granulocyte-monocyte progenitors, but stimulates erythrocyte progenitors.[58] The negative functions of NK-A can be explained by the production of haematopoietic suppressors, macrophage inflammatory protein 1α and transforming growth factor β.[23]

SP has been shown to be involved in haemorrhagic shock, a time at which the replacement of blood and immune cells[68 69] is urgently needed. During haemorrhagic shock, SP exhibits functional pleiotropism so as to maintain a balance in haematopoiesis. Hypoxia, which is linked to haemorrhagic shock, activates the transcription of hypoxia-inducible factor 1α, which interacts with the PPT-1 promoter to induce its expression.[68]

The induction of SP stimulates haematopoiesis so as to replace immune and blood cells.[68 69] During the period of haemorrhagic 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.[68]

SP is inactivated by the neutral endopeptidase of renal brush border.[70] Activity of the enzyme was also shown on the surface of neutrophiles,[71] lymphocytes,[72] enterocytes,[73] fibroblasts[74] and endothelial cells.[75]

Moreover, SP is believed to have an essential role as a modulator of synaptic transmission in sympathetic nerve fibres.[76] However, the sympathetic nervous system regulates the egress of stem and progenitor cells from their niche in BM.[77] This was shown in a model study using UDP-galactose ceramide galoctosyltransferase-deficient mice, which exhibited aberrant nerve conduction and displayed no stem and progenitor cells escape from BM following G-CSF administration. These results also raise the interesting

possibility that SP-derived alterations in sympathetic tone may explain the variability in mobilisation efficiencies among normal donors[78]; modulation of sympathetic outflow to the stem cell niche represents a novel strategy to increase the efficiency of haematopoietic stem and progenitor cell harvests for stem cell-based therapeutics.[77] Figure 1 summarises the data presented above.

RELATION OF SP TO NK-A AND HAEMOKININ-1 IN HAEMATOPOIESIS

In normal haematopoiesis, SP and NK-A, through the production of distinct cytokines, exert opposite effects with respect to proliferation of haematopoietic progenitors. The negative effects of NK-A on proliferation of BM progenitors suggest that NK-A might be protective to HSCs.[65] A protective role for NK-A is construed based on the predominant types of *TAC1* transcripts found in normal BM cells and in leukaemia cells. Normal BM stromal cells express transcript β, while leukaemic cells express only transcript α. While the former is capable of producing both NK-A and SP, the latter can only produce SP. This suggests that SP and NK-A might be able to regulate the proliferation of HSC through autocrine and/or paracrine mechanisms. Such a regulatory mechanism might not be possible in leukaemic cells, which produce only SP.[79–81] This argument is supported by reports showing an autocrine role for SP in proliferation of basophilic leukaemia cells.[82]

HK-1 has been also implicated in haematopoiesis.[25] A link between HK-1 and tachykinins derived from the *TAC1* gene is currently unclear. HK-1 regulates B and T lymphopoiesis[25 83] and directly affects the transition from pro-B to pre-B cells.[25] HK-1 promotes the survival and expansion of B cell lineage[84–86] and similarly facilitates T cell development at specific stages.[16] Regulation of the PPT-1 gene and/or functions of *TAC1* peptides could be altered, leading to BM disruption.

SUBSTANCE P AND MALIGNANT HAEMATOPOIESIS

Physiological haematopoiesis could be displaced by malignant tumours developing in BM. Neoplastic cells present there can originate directly from BM progenitors (leukaemia) or might be derived from metastatic cells of different solid tumours. Substance P plays an important role in pathogenesis of both groups of diseases.

Leukaemia

The leukaemic process involves abnormal differentiation and proliferation of neoplastically transformed stem cells. The cells infiltrate BM, leading to inhibited growth and differentiation of the remaining normal stem cells. The clinical signs reflect anaemia, leucopenia, low blood platelet level as well as involvement of tissues other than BM by the neoplastic process. Leukaemia can affect almost every organ, but lymph nodes, spleen, liver, central nervous system and skin in particular. Traditional classification of acute leukaemia was mainly based on morphological traits of cells examined by light microscopy. Subsequently, cytochemical and cytogenetic techniques were introduced; recently the armamentarium of techniques supporting morphological classification has been enriched by immunology and molecular biology. Since the control processes of normal haematopoiesis involve several pathways and several aspects, it is not easy to select a single in vivo factor, in this case SP, and to directly prove its significance for pathogenesis of acute leukaemia. However, the agent may play role in the pathogenesis of leukaemia since it is involved in the physiological control of haematopoiesis. The suggestion has been confirmed by results of the above mentioned studies on expression of *TAC1* transcripts.

An attempt was first made to clarify such a potential by monitoring the fate of patients in whom acute lymphoblastic leukaemia was diagnosed; SP expression was determined in blast cells, at the level of respective mRNA (classical in situ hybridisation) and the protein (immunocytochemistry). It was shown that in children diagnosed with acute lymphoblastic leukaemia of the B cell line phenotype, who were in the low risk group, expression of SP in blast cells before the start of treatment (and before introducing steroids in particular) represented an unfavourable prognostic index.[79] These children had a higher number of relapses, compared to children in the low risk group who showed no SP expression.

In the high risk group, the original expression of SP on BM blast cells had no prognostic significance. Interestingly, the expression of SP, in both high and low risk groups, showed no relationship to the risk of death resulting from progression of the disease. However, this might be explained by parallel involvement of several other variables, which together could result in death of the patients. Therefore, it proved impossible to select SP as the dominating factor.[79]

Similar results were obtained by De Giorgio *et al*.[87] Using flow cytometry, they estimated a strong SP immunoreactivity in lymphocyte-like cells derived from patients with acute non-lymphoblastic leukaemia, chronic myeloid leukaemia and B-chronic lymphoblastic leukaemia. In comparison to these neoplastically transformed cells, normal lymphocytes were, in general, negative or weakly positive for SP, suggesting that SP expression in neoplastic cells may be indicative of their activation state. Moreover, expression of SP in these types of leukaemia also indicated an unfavourable prognosis.

In our preliminary, not yet published studies, experimental 2-hour incubation of blast cells with an SP agonist was found to result in a significant increase of IL-1b concentration in BM sampled from children with acute lymphocytic leukaemia (ALL) who showed no SP expression in leukaemic cells. Incubation of the cells with an SP antagonist (spantide) resulted in control levels of IL-1b. However, in children with ALL and original expression of SP in blast cells, incubation of leukaemic cells with an SP agonist decreased by half the original IL-1b concentration. A similar concentration was obtained following incubation of the cells with spantide. The IL-1b concentration in the supernatant of the incubated cells from SP-positive children was twofold higher than that from SP-negative children. This might suggest that SP indirectly stimulated proliferation of leukaemic cells by enhancement of IL-1b synthesis in the cells. It should also be stressed that the phenomenon was observed within a relatively narrow range of SP concentrations of 10^{-10} to 10^{-8} mol/l.

Thus, the correlation between the original expression of SP in BM blast cells on the one hand and relapse of the disease in children in the low risk group on the other might suggest that SP is involved in the pathogenesis of acute lymphoblastic leukaemia in children. We have thus decided to also define SP expression in cases of bone marrow hypoplasia.

Bone marrow hypoplasia

BM hypoplasia appears as a morphological equivalent of clinically diagnosed haematopoietic insufficiency.[88] It is defined by the following laboratory values: haemoglobin <8.5 g/dl, mean corpuscular volume <88 fl, white blood cell count <2.0 g/l with neutrophil count <1.0 g/l, platelet count <50 g/l and reticulocytes $<0.1\%$.[88 89] BM aspirate reveals hypocellularity. Transient BM hypoplasia is usually caused by infections (both viral and bacterial) or by different chemical (including iatrogenic) and/or physical factors.[90 91] Inherited aplastic anaemia, or BM hypoplasia prior to the presence of non-haematopoietic tissue in BM (i.e., neuroblastoma metastases in BM), is a much more rare phenomenon. A considerable number of cases of BM hypoplasia may be subject to spontaneous remission and may not even be recognised. The

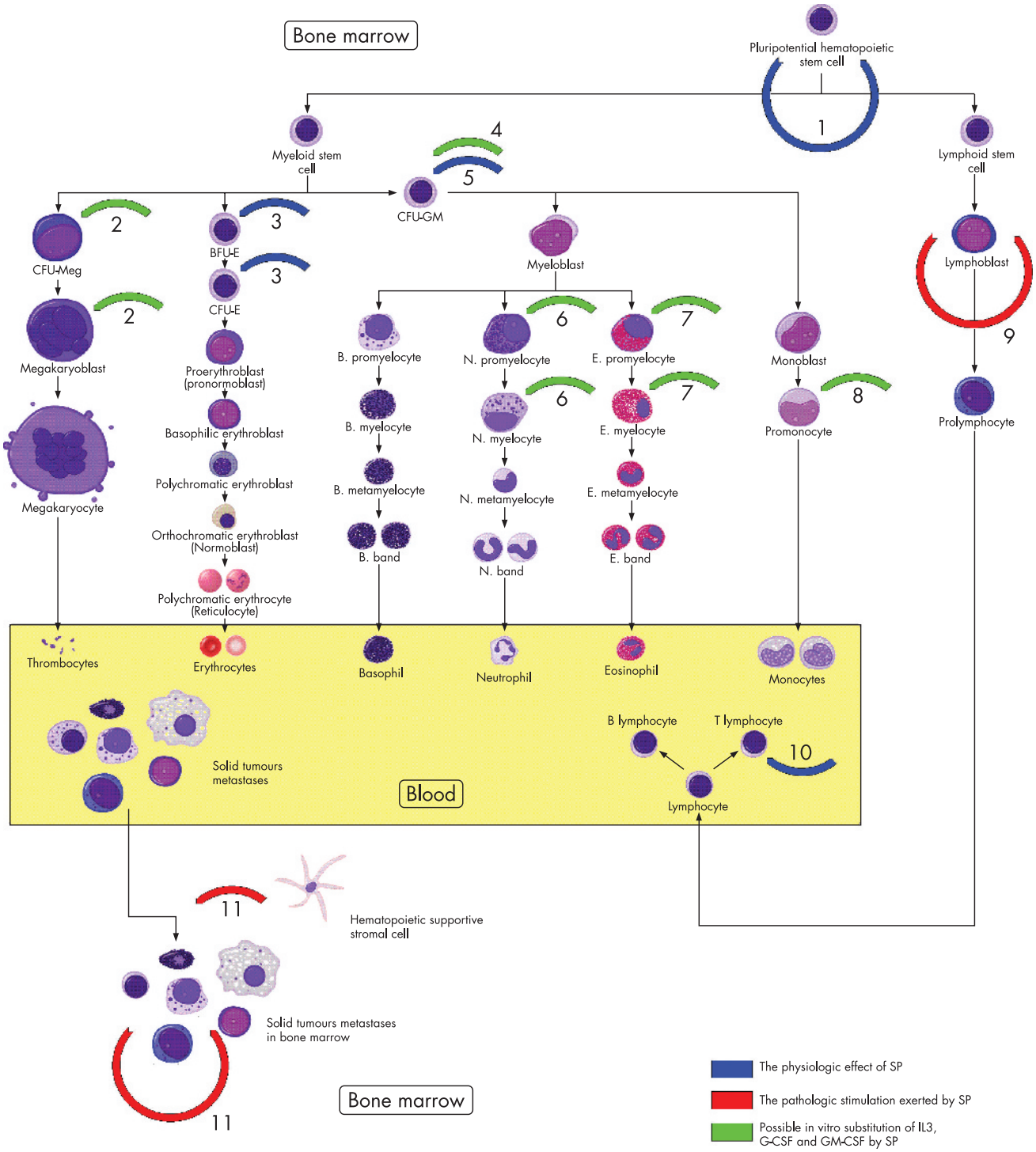

Figure 1 Diagrammatic representation of substance P (SP) effects on haematopoiesis and solid tumour metastases in bone marrow (BM). 1, Stimulation of BM pluripotent haematopoietic cells for self-renewal by release of stem cell factor.[55] 2, 4, 6–8, The potent role of SP in substitution of IL-3, G-CSF and GM-CSF in experimental studies.[52] 3, 5, Stimulatory effect of SP on proliferation of BFU-E, CFU-E and CFU-GM.[52 96] 9, Autostimulatory effect of SP on proliferation of neoplastically transformed lymphoblasts in acute leukaemia.[41 79 80] 10, SP augments proliferation of T lymphocytes.[46 47] 11, SP induces formation and homing of neuroblastoma and breast cancer metastases in bone marrow by autostimulation or direct influence of stromal cells.[97–108] CFU-GM, colony forming unit, granulocyte monocyte; CFU-Meg, colony forming unit megakaryocyte; CFU-E, colony forming unit erythroblast; BFU-E, burst forming unit erythroblast; IL, interleukin; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte monocyte colony stimulating factor.

first hospitalisation of a child with BM hypoplasia usually takes place after two or more ineffective courses of antibiotic therapy (for some undiagnosed chronic infection), followed by symptoms of anaemia and thrombocytopenia. In such cases BM hypoplasia can evolve into myelodysplastic syndrome, severe aplastic anaemia or neoplasia. Establishing the correct diagnosis usually requires several weeks during which the affected children are not treated but are monitored (laboratory evaluation

of peripheral blood, BM, chest radiographs, etc) at least once a month.

BM hypoplasia is not a malignant disease, but because of its close relation to the development of different types of acute leukaemia, could be regarded as a pre-neoplastic disorder. Acute leukaemia of either a lymphoblastic or non-lymphoblastic type that subsequently evolves into BM hypoplasia used to have a poor prognosis.[88] This may, at least in part, result from late introduction of a chemotherapy regimen. It is obvious that, in cases of neoplastic transformation, the type of chemotherapy used will depend on precise recognition of the tumour in question. Such treatment, however, could probably be introduced much earlier if expression of certain markers of early neoplastic transformation could be detected. The current obstacle is that the full list of possible markers is not universally accepted. However, some authors emphasise the fact that several of the cytokines that regulate physiological haematopoiesis may themselves represent potential risk factors of neoplastic transformation in BM hypoplasia.[31 32] The search for causes and aids to the diagnosis of BM hypoplasia involves taking a thorough history, a detailed physical examination and a number of laboratory tests, including microscopic analysis of BM, sampled by needle biopsy or during surgery, tests for infectious diseases and, occasionally, genetic studies on the patient and his/her family. Application of immunocytochemical techniques in the diagnosis of BM hypoplasia has already been tested.[92] However, attempts to use this technique have been restricted to differentiation of myelodysplastic syndromes from various grades of BM aplasia.[92–94] Among others, Ki67 antigen and proliferating cell nuclear antigen have been shown to be useful for the purpose.[94] In patients with BM aplasia, the markers could not be shown in nucleated cells, while in myelodysplastic syndromes the percentage of immunopositive cells has ranged from 20% to 60%.[94] These studies, however, have not attempted to determine the future trend of such BM hypoplastic lesions. Our selection of SP as an indicator of the potential trend of BM hypoplasia evolution was prompted by elucidation of its role, and the role of other neuropeptides, in the physiological control of haematopoiesis.

Our studies performed on BM of healthy individuals (who formed the control group) showed that the immunocytochemical expression of peripherally located SP (most probably coupled to NK-1R) on nucleated cells of the BM involved a 5% or lower fraction of the cells.[79 80] The results prompted us to perform analogous phenotyping of the material sampled from patients with clinical symptoms of BM hypoplasia.[95] In some, the proportion of SP-positive cells among all nucleated cells amounted to 67.6–95.8% (mean 81.5% cells for immunocytochemistry and 84.3% with in situ hybridisation) in the absence of neoplastic cells. Subsequent observation of these patients showed that they developed a proliferative disease of the BM. Throughout the time which preceded the neoplastic transformation, an increased number of SP-positive cells was noted, although the proportion of Ki67-positive cells was similar to control values. The neoplastic transformation was manifested by the appearance in BM of poorly differentiated cells with a positive reaction for Ki67 (39.6–49.8%). In line with the above, we suggest that presence of SP in B lymphocytes of normal appearance in hypoplastic BM becomes the additional (to nerve endings) source of this peptide, which may stimulate other neoplastically transformed cells to uncontrolled proliferation. Thus, SP might accelerate the already initiated development of leukaemia.[95] The origin of leukaemia, however, seems to be independent of SP expression.[96] The expression of SP in apparently normal BM lymphocytes before their neoplastic transformation could not predict death of the patient; this was related to the insufficient number of patients with neoplastic transformation and to different chemotherapy protocols used in the treatment of ALL and acute non-lymphocytic leukaemia. BM hypoplasia, followed by neoplastic transformation, frequently remains asymptomatic. Few patients receive specialist care before the symptoms of BM proliferative disease become evident. In these few patients the diagnosis of BM hypoplasia leads to consecutive check-ups, in the course of which immunocytochemical analysis of cellular inducers of differentiation on the surface of BM cells may provide a simple screening test, pointing to the potential trend of evolution of the lesion.

Solid tumours: neuroblastoma, breast cancer

Neuroblastoma is the third most common paediatric malignancy developing from neoplastically transformed ganglionic cells in the peripheral nervous system.[97] SP, together with other neuroendocrine markers such as neuropeptide Y, vasoactive intestinal peptide, somatostatin, and corticotrophin-releasing hormone are commonly expressed in ganglionic cells as well as in ganglioneuroblastoma samples[98] or metastatic cells in bone marrow.[99 100] Human neuroblastoma cell lines (SY5Y and CHP212) were found to express *TAC1* and the genes for NK-1R and NK-2R, at the levels of both mRNA and protein.[101] *TAC1* and NK receptors genes were also important for neuroblastoma cell proliferation and ability to establish metastatic foci in bone marrow.[101] The NK-1R deficient neuroblastoma cells did not proliferate when they were co-cultured with bone marrow stroma, which suggests that NK-1R signalling is important for the survival of neuroblastoma cells in the bone marrow.[101] It must be emphasised that expression of *TAC1* and the genes for NK-Rs is also a common event in other neuroblastoma endocrine-associated neoplasms such as breast cancer.[102 103]

Breast cancer is the most common malignant disease and the second leading cause of cancer mortality in women.[104] Compared to normal mammary epithelial cells and benign breast biopsy specimens, several breast cancer cell lines have shown increased expression of *TAC1* and NK-Rs.[101] Considering that *TAC1* peptides are haematopoietic modulators, the autocrine expression in breast cancer cells might explain their early integration in the bone marrow which is a preferred site of metastasis.[105] Moreover, bone marrow metastases of breast cancer correlate with poor prognosis and it is highly probable that metastatic cells settle in the bone marrow long before clinical detection of the tumour.[105] Studies by Rao *et al* showed that normal non-tumourigenic breast cells are not able to survive when co-cultured with bone marrow stroma.[106] This situation could be however be reversed when the above mentioned breast cells were genetically engineered to express *TAC1*. However, suppression of *TAC1* in breast cancer cells limited their malignancy and affected the process of bone marrow colonisation in knock-out mice.[106] Moreover, SP has an established stimulatory effect on the migration and metastatogenic phenotype in collagen matrix of MDA-MB-468, the human oestrogen receptor-negative breast carcinoma cell line; these effects can be prevented by certain NK-1R antagonists.[107] In this mechanism, SP is believed to up-regulate expression of $\alpha 2$ integrin (an essential adhesion receptor for collagen in migration), and down-regulate gelsolin (the tumour suppressor agent).[107] It is also possible that its total effect could be augmented by the potent role of SP in the initiation of angiogenesis.[108]

In line with the above, SP must be regarded not only as a growth factor in different tumour cells, but also as an regulating agent, promoting formation of metastases in bone marrow and impeding the physiological haematopoiesis. It must be emphasised that a similar mechanism involving SP could be also utilised in another cancers with a preference for bone marrow metastases: lung, prostate, and to a lesser extent, colon.[109]

Take-home messages

- Substance P (SP) has a role in both normal and pathological haematopoiesis. In the latter it interferes with the process of blast proliferation, acting as a tumour growth factor, or disturbs the physiological course of bone marrow cell maturation by promoting metastases of different solid tumours to bone marrow.
- The tachykinin system represents a potent therapeutic target, especially in tumours unresponsive to standard chemotherapy regimens. SP antagonists might be considered for use as anti-neoplastic drugs, for example by direct or indirect blocking of tumour cell proliferation through inhibition of growth factor production, including IL-1b synthesis.

ACKNOWLEDGEMENT

The authors wish to thank Professor Geoffrey Shaw for his help in English text editing.

Authors' affiliations

Michal Nowicki, Beata Kondraciuk, Department of Histology and Embryology, Poznan University of Medical Sciences, Poland
Danuta Ostalska-Nowicka, Department of Pediatric Cardiology and Nephrology, Poznan University of Medical Sciences, Poland
Bogdan Miskowiak, Department of Optometry and Biology of the Visual System, Poznan University of Medical Sciences, Poland

Competing interests: None.

REFERENCES

1 **Dąbrowski Z**, Tabarowski Z. History of discoveries of bone marrow and bone vascularization and innervation. In: Schoutens A, *et al*, eds. *Bone circulation and vascularization in normal and pathological conditions*. New York: Plenum Press, 1993:49–54.
2 **de Castro F**. Quelques observations sur l'intervention du systeme nerveux autonome dans l'ossification. Innervation du tissu osseux et de la moelle osseuse. Laboratoire de Recherches Biologiques de l'Universite de Madrid, 1930:215–44.
3 **Yamazaki K**, Allen TD. Ultrastructural morphometric study of efferent nerve terminals on murine bone marrow stromal cells, and the recognition of a novel anatomical unit: 'the neuro-reticular complex'. *Am J Anatomy* 1990;**187**:261–76.
4 **Imai S**, Tokunaga Y, Maeda T, *et al*. Calcitonine-gene related peptide, substance P and tyrosine hydroxylase-immunoreactive innervation of rat bone marrows: an immunohistochemical and ultrastructural investigation on possible efferent and afferent mechanism. *J Orthop Research* 1997;**15**:133–40.
5 **Ahmed M**, Bjurholm A, Kreibergs A, *et al*. Neuropeptide Y, tyrosine hydroxylase and vasoactive intestinal polypeptide-immunoreactive nerve fibers in the vertebral bosies, discs, dura mater and spinal ligaments of the rat lumbar spine. *Spine* 1993;**18**:268–73.
6 **Bianco P**, Riminucci M, Gronthos S, *et al*. Bone marrow stromal stem cells: nature, biology, and potential applications. *Stem Cells* 2001;**19**:180–92.
7 **Zon LI**. Developmental biology of hematopoiesis. *Blood* 1995;**86**:2876–91.
8 **Kondo M**, Wagers AJ, Manz MG, *et al*. Biology of hematopoietic stem cells and progenitors: implications for clinical application. *Ann Rev Immunol* 2003;**21**:759–806.
9 **Deans RJ**, Moseley AB. Mesenchymal stem cells: biology and potential clinical uses. *Exp Hematol* 2000;**28**:875–84.
10 **Arai F**, Ohneda O, Miyamoto T, *et al*. Mesenchymal stem cells in perichondrium express activated leukocyte cell adhesion molecule and participate in bone marrow formation. *J Exp Med* 2002;**195**:1549–63.
11 **Caplan AI**, Bruder SP. Mesenchymal stem cells: building blocks for molecular medicine in the 21st century. *Trends Mol Med* 2001;**7**:259–64.
12 **Kaushansky K**, Karplus AP. Hematopoietic growth factors: understanding functional diversity in structural terms. *Blood* 1993;**82**:3229–36.
13 **Greco SJ**, Corcoran KE, Cho KJ, *et al*. Tachykinins in the emerging immune system: relevance to bone marrow homeostasis and maintenance of hematopoietic stem cells. *Front Biosci* 2004;**9**:1782–93.
14 **Fras C**, Kravetz P, Mody DR, *et al*. Substance P-containing nerves within the human vertebral body: an immunohistochemical study of the basiverterbral nerve. *Spine J* 2003;**3**:63–7.
15 **Tabarowski Z**, Gibson-Berry K, Felten SY. Noradrenergic and peptidergic innervation of the mouse femur bone marrow. *Acta Histochem* 1996;**98**:453–7.
16 **van Hagen PM**, Krenning EP, Kwekkeboom DJ, *et al*. Somatostatin and the immune and haematopoietic system: a review. *Eur J Clin Invest* 1994;**24**:91–9.
17 **Zhang Y**, Paige CJ. T-cell developmental blockage by tachykinins antagonists and the role of hemokinin 1 in T lymphopoiesis. *Blood* 2003;**102**:2165–72.
18 **Patacchini R**, Lecci A, Holzer P, *et al*. Newly discovered tachykinins raise new questions about their peripheral roles and the tachykinin nomenclature. *Trends Pharmacol Sci* 2004;**25**:1–3.
19 **Quartara L**, Maggi CA. The tachykinin NK1 receptor: part II. Distribution and pathophysiological roles. *Neuropeptides* 1998;**32**:1–49.
20 **Beaujouan JC**, Torrens Y, Saffroy M, *et al*. A 25 year adventure in the field of tachykinins. *Peptides* 2004;**25**:339–57.
21 **Pennefather JN**, Lecci A, Candenas ML, *et al*. Tachykinins and tachykinin receptors: a growing family. *Life Sci* 2004;**74**:1445–63.
22 **Rameshwar P**, Gascón P. Hematopoietic modulation by the tachykinins. *Acta Haematol* 1997;**98**:59–64.
23 **Rameshwar P**, Poddar A, Gascón P. Hematopoietic regulation mediated by interactions among the neurokinins and cytokines. *Leuk Lymphoma* 1997;**28**:1–10.
24 **Morteau O**, Lu B, Gerard C, *et al*. Hemokinin I is a full agonist at the substance P receptor. *Nat Immunol* 2001;**2**:1088–92.
25 **Zhang Y**, Lu L, Furlonger C, *et al*. Hemokinin is a hematopoietic specific tachykinin that regulates B lymphopoiesis. *Nat Immunol* 2000;**1**:392–7.
26 **Page NM**, Bell NJ, Gardiner SM, *et al*. Characterization of the endokinins: human tachykinins with cardiovascular activity. *Proc Natl Acad Sci USA* 2003;**100**:6245–50.
27 **Milne CD**, Fleming HE, Zhang Y, *et al*. Mechanism of selection mediated by interleukin-7, the preBCR, and hemokinin-1 during B-cell development. *Immunol Rev* 2004;**197**:75–88.
28 **Pinto FM**, Almeida TA, Hernandez M, *et al*. mRNA expression of tachykinins and tachykinin receptors in different human tissues. *Eur J Pharmacol* 2004;**494**:233–9.
29 **Donnelly D**, Maudsley S, Gent JP, *et al*. Conserved polar residues in the transmembrane domain of the human tachykinin NK2 receptor: functional roles and structural implications. *Biochem J* 1999;**339**:55–61.
30 **Aronin N**. Substance P and the tachykinins. Endocrinology and metabolism. In: Becker KL, ed. *Principles and practice of endocrinology and metabolism*. Philadelphia: JB Lippincott Company, 1990:1309–22.
31 **Maggio J**. Tachykinins. *Ann Rev Neurosci* 1990;**11**:13–26.
32 **Pernow B**. Substance P. *Pharmacol Rev* 1983;**35**:85–98.
33 **Holzer P**. Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene-related peptide and other neuropeptides. *Neuroscience* 1988;**24**:39–44.
34 **Lembeck F**, Holzer P. Substance P as neurogenic mediator of antidromic vasodilatation and neurogenic plasma extravasation. *Naunyn-Schmiedeberg's Arch Pharmacol* 1979;**310**:175–80.
35 **Rang H**, Bevan S, Dray A. Chemical activation of nociceptive peripheral neurones. *Br Med Bull* 1991;**47**:534–43.
36 **Calvo C**, Chavanel G, Senik A. Substance P enhances IL-2 expression in activated human T-cells. *J Immunol* 1992;**148**:3498–513.
37 **Ansel J**, Brown J, Payan D, *et al*. Substance P selectively activates TNF-α gene expression in murine mast cell. *J Immunol* 1993;**150**:4478–83.
38 **Stead R**, Bienenstock J, Stanisz A. Neuropeptide regulation of mucosal immunity. *Immunol Rev* 1987;**100**:33–43.
39 **Shanahan F**, Anton P. Neuroendocrine modulation of the immune system. *Dig Dis Sci* 1988;**33**:123–44.
40 **Savino W**, Dardenne M. Immuno-neuroendocrine interactions. *Immunol Today* 1995;**16**:318–29.
41 **Sirinek L**, O'Dorisio M. Modulation of immune function by intestinal neuropeptides. *Acta Oncol* 1991;**30**:509–18.
42 **Bost K**, Breeding S, Pascual D. Modulation of the mRNAs encoding substance P and its receptor in rat macrophages by LPS. *Reg Immunol* 1992;**4**:105–12.
43 **Linnik D**, Moskowitz A. Identification of immunoreactive substance P in human and other mammalian endothelial cells. *Peptides* 1989;**10**:957–63.
44 **Ziche M**, Morbidelli L, Pacini M, *et al*. NK-1 receptors mediate the proliferative response of human fibroblasts to tachykinins. *Br J Pharmacol* 1990;**100**:11–23.
45 **Rameshwar P**, Gascon P. Substance P (SP) mediates production of stem cell factor and interleukin-1 in bone marrow stroma: potential autoregulatory role for these cytokines in SP receptor expression and induction *Blood* 1995;**86**:482–90.
46 **Payan D**, Brewster D, Goetzl E. Specific stimulation of human T lymphocytes by substance P. *J Immunol* 1983;**131**:1613–22.
47 **Scicchitano R**, Biennestock J, Stanisz A. In vivo immunomodulation by the neuropeptide substance P. *Immunology* 1988;**63**:733–42.
48 **Payan D**. Receptor-mediated mitogenic effect of substance P on cultured smooth muscle cells. *Biochem Biophys Res Commun* 1985;**130**:104–13.
49 **Woll P**, Rosengurt E. Neuropeptides as growth regulators. *Br Med Bull* 1989;**45**:492–9.
50 **Nilsson G**, Pernow B, Fisher G, *et al*. Presence of substance P- like immunoreactivity in plasma from man and dog. *Acta Physiol Scand* 1975;**94**:542–51.
51 **Lotz M**, Carson D, Vaughan J. Substance P activation of rheumatoid synoviocytes: neural pathway in pathogenesis of arthritis. *Science* 1987;**235**:893–905.
52 **Rameshwar P**, Ganea D, Gascón P. In vitro stimulatory effect of substance P on hematopoiesis. *Blood* 1993;**81**:391–8.
53 **Cozens P**, Rowe F. Substance P is a potent inducer of TNF and IL-1 secretion by macrophages. *Immunology* 1987;**175**:7–16.
54 **Laurenzi M**, Persson M, Dalsgaard C, *et al*. The neuroendopeptide substance P stimulates production of interleukin 1 in human blood monocytes: activated cells

are preferentially influenced by the neuropeptide. *Scand J Immunol* 1990;**31**:529–538.
55 **Rameshwar P**, Gascón P, Ganea D. Immunoregulatory effects of neuropeptides. Stimulation of interleukin- 2 production by substance P. *J Neuroimmunol* 1992;**37**:65–74.
56 **Wagner F**, Fink R, Hart R, *et al*. Substance P enhances interferon- gamma production by human peripheral blood mononuclear cells. *Regul Pept* 1987;**19**:355–361.
57 **Buzby J**, Knoppel E, Cairo M. Coordinate regulation of Steel factor, its receptor (kit) and cytoadhesion molecule (ICAM-1 and ECAM-1) mRNA expression in human vascular endothelial cellsof differing origins. *Exp Hematol* 1994;**22**:122–31.
58 **Felten S**, Felten D, Bellinger D, *et al*. Noradrenergic and peptidergic innervation of lymphoid organs. *Neuroimmunoendocrinology* 1992;**25**:67–77.
59 **Pascual D**, Bost K. Substance P production by P388D1 macrophages: a possible autocrine function for this neuropeptide. *Immunology* 1990;**71**:52–77.
60 **Weinstock J**, Blum A, Walder J, *et al*. Eosinophils from granulomas in murine Schistosomatosis mansoni produce substance P. *J Immunol* 1988;**141**:961–6.
61 **Weinstock J**, Blum A. Tachykinin production by granuloma eosinophils in murine Schistosomatosis mansoni. *J Immunol* 1989;**142**:3256–61.
62 **Walsh D**, Wharton J, Blake D, *et al*. Neural and endothelial regulatory peptides, their possible involvement in inflammation. *Int J Tissue React* 1992;**14**:101–12.
63 **Greeno E**, Mantych P, Vercellotti G, *et al*. Functional neurokinin 1 receptors for substance P are expressed by human vascular endothelium. *J Exp Med* 1993;**177**:1269–78.
64 **Payan D**, Brewster D, Missirian-Bastian A, *et al*. Substance P recognition by a subset of human T-lymphocytes. *J Clin Invest* 1984;**74**:1532–7.
65 **Rameshwar P**, Gascón P. Induction of negative hematopoietic regulators by neurokinin-A in bone marrow stroma. *Blood* 1996;**88**:98–106.
66 **Rameshwar P**. Substance P: a regulatory neuropeptide for hematopoiesis and immune functions. *Clin Immunol Immunopathol* 1997;**85**:129–33.
67 **Rameshwar P**, Gascón P. Neural regulation of hematopoiesis by the tachykinins. *Mol Biol Hemat* 1996;**5**:463.
68 **Qian J**, Ramroop K, McLeod A, *et al*. Induction of hypoxia-inducible factor-1α and caspase-3 in hypoxic bone marrow stroma is negatively regulated by the delayed production of substance P. *J Immunol* 2001;**167**:4600–8.
69 **Quinlan D Jr**, Rameshwar P, Qian J, *et al*. Effect of hypoxia on the hematopoietic and immune modulator preprotachykinin-1. *Arch Surg* 1998;**133**:1328–34.
70 **Kerr M**, Kenny A. The purification and specificity of a neutral endopeptidase from rabbit kidney brush border. *Biochem J* 1974;**137**:477–81.
71 **Skidgel R**, Jackman H, Erdos E. Metabolism of substance P and bradykinin by human neutrophils. *Biochem Pharmacol* 1991;**41**:1335–41.
72 **Tran-Paterson R**, Willard H, Leterte M. The common acute lymphoblastic leukemia antigen (neutral endopeptidase) gene is located on human chromosome 3. *Cancer Genet Cytogenet* 1989;**42**:129–41.
73 **Iwamoto I**, Ueki I, Nadel J. Effect of neutral endopeptidase inhibitors on 3H-substance P binding in rat ileum. *Neuropeptides* 1988;**11**:185–96.
74 **Johnson A**, Ashton J, Schultz W, *et al*. Neutral metalloendopeptidase from rabbit kidney brush border. *Am Rev Respir Dis* 1985;**132**:564–73.
75 **Erdos E**, Skidgel R. Neutral endopeptidase 24.11 (enkephalinase) and related regulators of peptide hormones. *FASEB J* 1989;**3**:145–56.
76 **Jobling P**, Messenger JP, Gibbins IL. Differential expression of functionally identified and immunohistochemically identified NK(1) receptors on sympathetic neurons. *J Neurophysiol* 2001;**85**:1888–98.
77 **Katayama Y**, Battista M, Kao WM, *et al*. Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. *Cell* 2006;**124**:407–21.
78 **Korbling M**, Huh YO, Durett A, *et al*. Allogeneic blood stem cell transplantation: peripheralization and yield of donor-derived primitive hematopoietic progenitor cells (CD34+ Thy-1dim) and lymphoid subsets, and possible predictors of engraftment and graft-versus-host disease. *Blood* 1995;**86**:2842–8.
79 **Nowicki M**, Miskowiak B. Substance P—a potent risk factor in childhood lymphoblastic leukaemia. *Leukemia* 2003;**6**:1096–9.
80 **Nowicki M**, Miskowiak B, Ostalska-Nowicka D. Detection of substance P and its mRNA in human blast cells in childhood lymphoblastic leukaemia using immunocytochemistry and in situ hybridisation. *Folia Histochem Cytobiol* 2003;**41**:33–6.
81 **Rameshwar P**, Oh HS, Yook C, *et al*. Substance P-fibronectin cytokine interactions in myeloproliferative disorder with bone marrow fibrosis. *Acta Haematol* 2003;**109**:1–10.
82 **Suzuki R**, Furuno T, McKay DM, *et al*. Direct neurite-mast cell communication in vitro occurs via the neuropeptide substance P. *J Immunol* 1999;**163**:2410–15.
83 **Zhou D**, Kusnecov AW, Shurin MR, *et al*. Exposure to physical and psychological stressors elevates plasma interleukin 6: relationship to the activation of hypothalamic-pituitary-adrenal axis. *Endocrinology* 1993;**133**:2523–30.
84 **Coa YA**, Wagers AJ, Beilhack A, *et al*. Shifting foci of hematopoiesis during reconstitution from single stem cells. *Proc Natl Acad Sci USA* 2004;**101**:221–6.
85 **Maestroni GJM**. Adrenergic regulation of haematopoiesis. *Pharmacol Res* 1995;**32**:249–53.
86 **Morrison SJ**, Shah NM, Anderson DJ. Regulatory mechanisms in stem cell biology. *Cell* 1997;**88**:287–98.
87 **De Giorgio R**, Tazzari PL, Barbara G, *et al*. Detection of substance P immunoreactivity in human peripheral leukocytes. *J Neuroimmunol* 1998;**82**:175–81.
88 **Stockman JA**. Aplastic anemia (in Polish). In: Behrman RE, Kliegman R, Jenson AB, eds. *Nelson textbook of pediatrics*. 14th edn. Warsaw: PWN, 1996:1426–8.
89 **Alter BP**, Potter NU. Classification and aetiology of the aplastic anaemias. *Clin Haematol* 1978;**7**:431–9.
90 **Williams DM**, Lynch RE, Cartwright GE. Drug-induced aplastic anemia. *Semin Hematol* 1973;**10**:195–201.
91 **Baranski B**, Armstrong G, Truman JT, *et al*. Epstein-Barr virus in the bone marrow of patients with aplastic anemia. *Ann Intern Med* 1988;**109**:695–704.
92 **Elias J**, Gown A, Nakamura R. Special report: Quality control in immunohistochemistry. *Am J Clin Pathol* 1989;**92**:836–43.
93 **Sadahira Y**, Wada H, Manabe T, *et al*. Immunohistochemical assessment of human bone marrow macrophages in hematologic disorders. *Pathol Int* 1999;**49**:626–32.
94 **Kitagawa M**, Kamiyama R, Kasuga T. Expression of the proliferating cell nuclear antigen in bone marrow cells from patients with myelodysplastic syndromes and aplastic anemia. *Hum Pathol* 1993;**24**:359–63.
95 **Nowicki M**, Ostalska-Nowicka D, Konwerska A, *et al*. The predicting role of substance P in the neoplastic transformation of the hypoplastic bone marrow. *J Clin Pathol* 2006;**59**:935–41.
96 **Kang HS**, Trzaska KA, Corcoran K, *et al*. Neurokinin receptors: relevance to the emerging immune system. *Arch Immunol Ther Exp* 2004;**52**:328–47.
97 **Weinstein JL**, Katzenstein HM, Cohn SL. Advances in the diagnosis and treatment of neuroblastoma. *Oncologist* 2003;**8**:278–92.
98 **Kimura N**, Yamamoto H, Okamoto H, *et al*. Multiple-hormone gene expression in ganglioneuroblastoma with water diarrhea, hypokalemia, and achlorhydria syndrome. *Cancer* 1993;**71**:2841–6.
99 **Nowicki M**, Miskowiak B. Comparison of the cell immunophenotype of metastatic and primary foci in stage IV-S neuroblastoma. *Folia Histochem Cytobiol* 2002;**40**:297–303.
100 **Nowicki M**, Ostalska-Nowicka D, Miskowiak B. Prognostic value of stage IV neuroblastoma metastatic immunophenotype in the bone marrow: preliminary report. *J Clin Pathol* 2006;**59**:150–2.
101 **Mukerji L**, Ramkssoon SH, Reddy KKR, *et al*. Autocrine proliferation of neuroblastoma cells is partly mediated through neurokinin receptors: relevance to bone marrow metastasis. *J Neurooncol* 2005;**71**:91–8.
102 **Singh D**, Joshi DD, Hameed M, *et al*. Increased expression of preprotachykinin-I and neurokinin receptors in human breast cancer cells: implications for bone marrow metastasis. *Proc Natl Acad Sci USA* 2000;**97**:388–93.
103 **Oh HS**, Moharita A, Potian JG, *et al*. Bone marrow stroma influences transforming growth factor-beta production in breast cancer cells to regulate c-myc activation of the preprotachykinin-I gene in breast cancer cells. *Cancer Res* 2004;**64**:6327–36.
104 **Landis SH**, Murray T, Bolden S, *et al*. Cancer statistics, 1999. *CA Cancer J Clin* 1999;**49**:8–31.
105 **Gluck S**. Autologous transplantation for patients with advanced breast cancer with emphasis on bony metastasis. *Can J Oncol* 1995;**5**(Suppl 1):58–62.
106 **Rao G**, Patel PS, Idler SP, *et al*. Facilitating role of preprotachykinin-I gene in the integration of breast cancer cells within the stromal compartment of the bone marrow: a model of early cancer progression. *Cancer Res* 2004;**15**:2874–81.
107 **Lang K**, Drell TL 4th, Lindecke A, *et al*. Induction of a metastatogenic tumor cell type by neurotransmitters and its pharmacological inhibition by established drugs. *Int J Cancer* 2004;**112**:231–8.
108 **Kato H**, Shichiri M, Marumo F, *et al*. Adrenomedullin as an autocrine/paracrine apoptosis survival factor for rat endothelial cells. *Endocrinology* 1997;**138**:2615–20.
109 **Palma C**. Tachykinins and their receptors in human malignancies. *Curr Drug Targets* 2006;**8**:1043–52.