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Somatostatin receptors in the haematopoietic system

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Introduction

Multiple interactions exist between the immune, haematopoietic, endocrine and nervous systems (1,2). The bi-directional communication between the immune/haematopoietic and nervous systems is mediated by complex mechanisms involving multiple soluble factors (e.g. neuropeptides, neurotrophic factors, neurotransmitters and cytokines) produced by each system (3–5). Examples of such factors are: the neurotransmitter neuropeptide Y (6) produced by megakaryocytes (7), substance P, which enhances the proliferation of primitive bone marrow cells and progenitors (4), and nerve growth factor (NGF), which contributes to differentiation of human basophils (8) and stimulates the release of inflammatory mediators from these cells (9).

The bone marrow is the major site of haematopoiesis in adults. All blood cells descend from pluripotent haematopoietic stem cells, which develop in lineage-committed progenitors. These committed progenitors expand and differentiate towards functional end cells. Haematopoiesis is controlled by a complex cytokine network in combination with cellular signals provided by cell-to-cell contact with stromal elements within the bone marrow. A number of studies have demonstrated that somatostatin inhibits proliferation of lymphoid and haematopoietic cells (10). However, little is known of the effects of somatostatin on primary haematopoietic stem cells and progenitor cells. This brief overview will focus on recent insights into the expression and functional significance of somatostatin receptors (SST) in the haematopoietic system.

Haematopoiesis

Development

During mammalian embryogenesis, the haematopoietic system is formed from mesodermally derived cells localised in the aorta–gonad–mesonephros region and the yolk-sac and are predominantly pluripotent haematopoietic stem cells. At a later stage in the development of the foetus, haematopoiesis takes place in the liver and subsequently haematopoiesis shifts to the spleen and the bone marrow. The spleen then gradually becomes a less important haematopoietic organ and, at birth, haematopoiesis in humans is almost exclusively situated in the bone marrow (11).

Haematopoiesis is a strictly regulated process. All blood cells are derived from a small population of pluripotent stem cells that are capable of self-renewal and differentiation towards distinct lineage-committed progenitor cells. These committed progenitor cells can undergo proliferation followed by terminal differentiation into the different mature blood cell types. Blood cells have a finite lifespan and must be replaced constantly throughout life. In addition to this requirement to maintain circulating cell numbers, it is also necessary to respond to host challenges with appropriately increased output of the specific cell types required. Finally, it is necessary to down regulate output when the response is no longer required. This continuous production is tightly balanced and regulated essentially by two mechanisms. Stromal cells and extracellular matrix in the bone marrow provide a suitable microenvironment required for haematopoietic cell development. In addition, a network of cytokines and haematopoietic growth factors (HGF) specifically controls the proliferation, differentiation, survival, and function of different haematopoietic cells. This network is particularly important under stress conditions, such as infection or bleeding, when rapid increases in specific blood cell types are needed.

Migration and homing of cells

During foetal development, the multipotential and self-renewing haematopoietic stem cells migrate from the foetal liver to the bone marrow. This phenomenon of targeted migration *via* the circulation to a specific tissue is referred to as 'homing' (12). Regulation of progenitor cell mobilisation and homing is a complex process, involving adhesion molecules, paracrine cytokines, and chemokines (Fig. 1). Two types of migration can be distinguished. Chemoattraction is the unidirectional movement of cells towards a positive gradient of a compound, whereas chemokinesis reflects activation of cell motility and an induction of cell migration in a random direction. Chemokines comprise a large number of structurally related proteins that regulate migration and activation of leukocytes through G-protein-coupled cell-surface receptors (14). The first chemoattractant reported for human $CD34^+$ progenitor

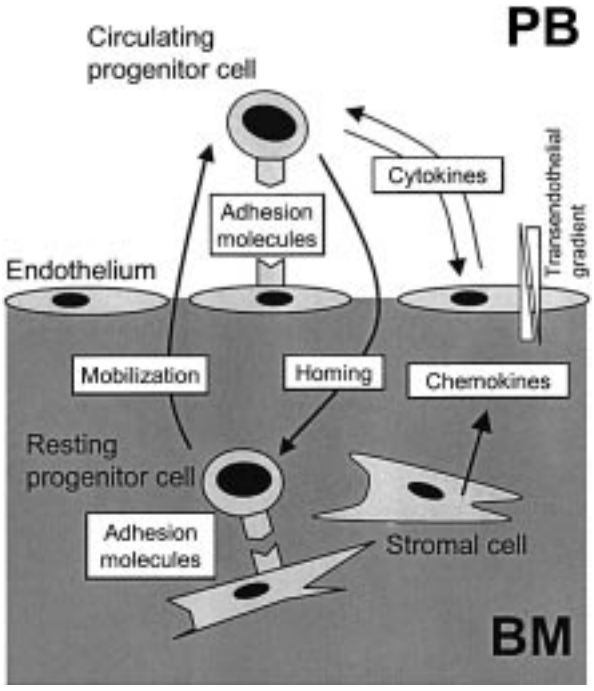

Figure 1 Trafficking of haematopoietic progenitor cells. Mobilisation and homing of haematopoietic progenitor cells are multifactorial processes that involve interactions via adhesion molecules, chemokines, and paracrine cytokines. Transendothelial migration most probably has a role in haematopoietic progenitor cell trafficking. Adhesion molecules expressed on progenitor and bone marrow endothelial cells may regulate transition from resting to the circulating progenitor cell compartment and *vice versa*. Chemokines produced in the bone marrow stroma build up transendothelial gradients that may either support or inhibit migration of progenitor cells across the endothelial layer. In addition, endothelial cells can produce cytokines that influence proliferation and motility of progenitors, and haematopoietic progenitor cells may also produce cytokines that act on endothelial cells. PB: peripheral blood, BM: bone marrow. Redrafted with permission from (13).

cells is stromal-cell-derived factor-1 (SDF-1) (15). This chemokine, produced by bone marrow stromal cells, is a ligand for the chemokine receptor CXCR4. *In vitro*, SDF-1 elicits maximal transendothelial migration of ~25% of the $CD34^+$ population 3 h after exposure. SDF-1 is also a chemoattractant for human lymphocytes and monocytes (16). Furthermore, SDF-1 and its receptor CXCR4 were found to be critical for murine bone marrow engraftment by human severe combined immunodeficient (SCID) repopulating stem cells (17). Besides SDF-1, stem cell factor (SCF) has been shown to elicit some chemotaxis/chemokinesis on mouse progenitor cells (18). However, the percentage of cells migrating in response to SCF was lower, and maximal migration occurred much later (8–24 h) than seen in response to SDF-1 (2–4 h), suggesting that SCF-induced migration might be attributable to indirect effects.

Somatostatin receptors in the haematopoietic system

The presence of SSTs has been demonstrated in human lymphoid tissues, lymphoid cell lines and peripheral blood cells (19–26). Both SST_2 and SST_3 transcripts have been detected in freshly isolated human thymocytes (27). In contrast, human peripheral blood B- and T-lymphocytes express only SST_3. Monocytes express SST_2 upon activation, for example by lipopolysaccharides (28). Until now, no data have been available on SST expression on haematopoietic precursors. We therefore examined the expression of SST subtypes on human bone marrow cells by RT-PCR and by flow cytometric analysis using fluorescent somatostatin. Of the five SST subtypes, SST_2 is exclusively expressed in human bone marrow cells. Interestingly, SST_2 are present on a small subset (<1%) of cells. Immunophenotypic analysis showed that this subpopulation represents the $CD34^+$ fraction, with the $CD117^+$ (c-kit^+) subset of $CD34^+$ cells showing the greatest expression (29). This fraction comprises the most primitive stages of haematopoietic differentiation, including the pluripotent stem cells and early lineage-committed progenitor cells.

Haematopoietic malignancies: lymphomas and leukaemias

SSTs are also present on cells derived from several haematological malignancies (20, 30–32). SSTs have been detected *in vivo* by scintigraphy using radiolabelled somatostatin analogues in both T and B non-Hodgkin's lymphoma and Hodgkin's disease (20, 30, 33, 34). Somatostatin receptor autoradiography, in which tissue sections are incubated with isotope-labelled somatostatin or somatostatin analogues, has been applied to demonstrate the presence of somatostatin binding sites in biopsy specimens from malignant lymphomas (20). Using similar methods, somatostatin binding sites have been detected on acute lymphoblastic leukaemia and acute myeloid leukaemia (AML) (31). Receptors for somatostatin have also been detected on lymphoblastic leukaemia by using a fluorescent somatostatin (32). We have recently demonstrated that, as in normal bone marrow, only SST subtype 2 is expressed on AML cells. Importantly, in contrast to normal human bone marrow, the SST_2 expression is not restricted to $CD34^+/CD117^+$ in AML cells (S P M A Oomen, unpublished observations), which might suggest that SST_2 expression in AML is not as tightly regulated as in normal cells.

Function of SST in the haematopoietic system

Inhibition of proliferation

Somatostatin is best known for its inhibitory actions on hormone secretion and cell proliferation. Moderate

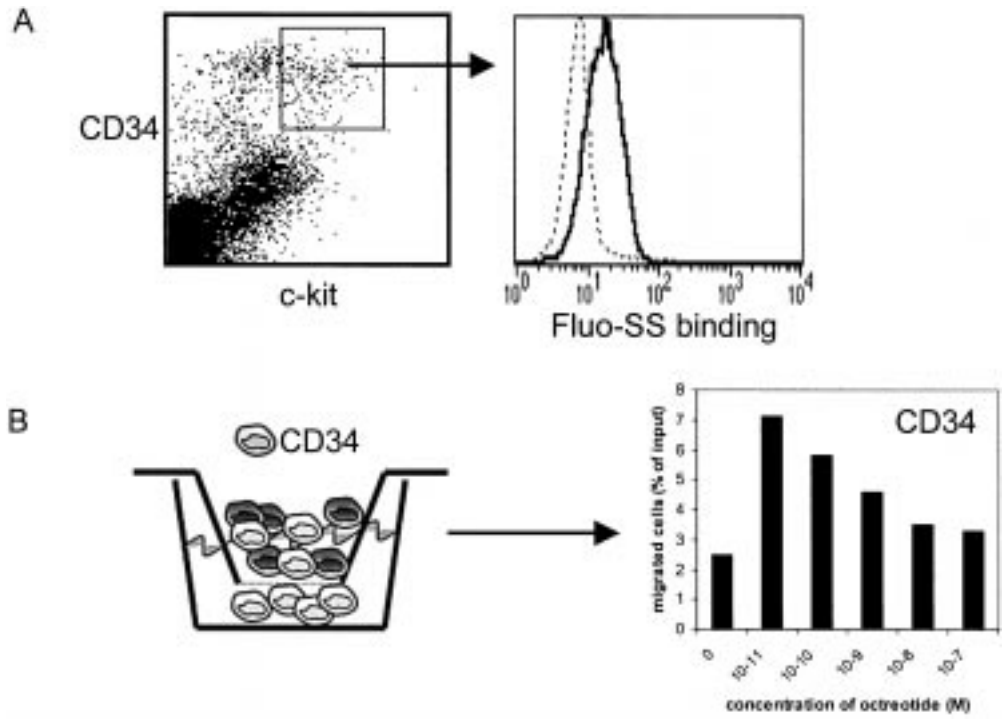

Figure 2 Octreotide induces migration of human $CD34^+$ cells. (A) Analysis of Fluo-somatostatin (SS) binding to $CD34^+/CD117^+$ cells. A window was set to select cells on the basis of CD34/CD117 expression. Cells were incubated in the absence (solid line) or presence (broken line) of a 100-fold excess of $(D\text{-}Trp^8)$-somatostatin-14. (B) Migration assay of $CD34^+$ cells in response to indicated concentrations of octreotide: migrated cells as a percentage of input cells.

inhibitory effects of somatostatin and octreotide on the *in vitro* proliferation of AML cells have been reported earlier (31). The antiproliferative effect of somatostatin on AML cells depended on the type of haematopoietic growth factor used to induce proliferation. The inhibitory effects were most prominent when AML cells had been stimulated with granulocyte colony-stimulating factor (G-CSF). The growth inhibitory effects of somatostatin have been attributed to binding and activation of a subclass of protein tyrosine phosphatase (PTPase) enzymes (35–37). Src homology domain-containing protein-tyrosine phosphatase-1 (SHP-1), previously referred to as haematopoietic cell phosphatase, has been postulated as the PTPase responsible for SST_2-mediated inhibitory growth signalling (35). We have recently studied the mechanisms underlying somatostatin responses in a myeloid cell line (mouse 32D cells) stably expressing SST_2 and G-CSF receptors. In this model, somatostatin and octreotide reduced G-CSF-induced proliferation by approximately 50%. Incubation with octreotide significantly increased the activity of SHP-1 in these cells (S P M A Oomen, unpublished observations). Because SHP-1 does not bind directly to the G-CSF receptor complex (38,39), these data fit into a hypothetical model in which SST_2 recruits SHP-1 to the plasma membrane, where it can down modulate proliferative signals from the G-CSF receptor.

Migration

Only a few studies have dealt with effects of somatostatin on cell migration (chemotaxis) of monocytes and the results have been contradictory (38, 40, 41). In view of the observation that SST_2 is expressed on $CD34^+$ bone marrow cells and because other $G_{\alpha i}$-coupled receptors such as CXCR4 and IL-8 receptor have been implicated in the control of haematopoietic cell migration (15, 42, 43), we determined whether somatostatin induces migration of primitive haematopoietic cells. We found that octreotide indeed acts as a potent pro-migratory stimulus for $CD34^+$ bone marrow cells (29) (Fig. 2). Using the 32D cell line model referred to above, we were able to show that octreotide acts predominantly as a chemoattractant, but has also some chemokinetic activity (Fig. 3). Finally, we observed that somatostatin also induced migration of AML cells (S P M A Oomen, unpublished observations). These data suggest that somatostatin may have an effect on the homing and migration of normal and AML cells *in vivo*, with possible implications for the clinical application of somatostatin and its analogues.

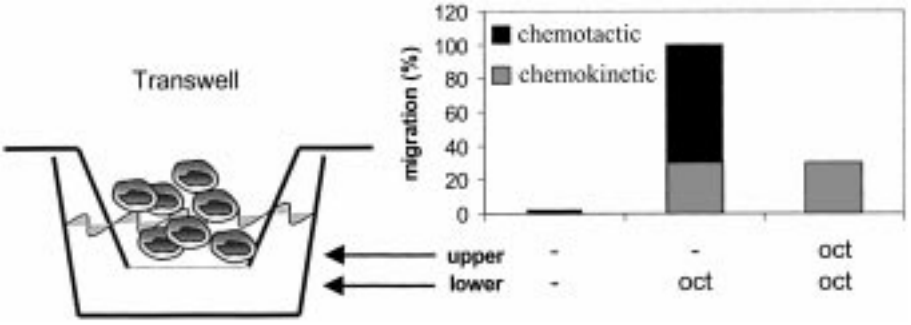

Figure 3 Chemotactic and chemokinetic activity of octreotide. Octreotide 10^{-9} mol/l (oct) was added to upper or lower chambers, or both, of a transwell as indicated. Data are expressed as the percent migration to a positive gradient (100%). These experiments were performed with a model for haematopoietic precursor cells.

Somatostatin in the haematopoietic system

Because somatostatin is rapidly degraded in the circulation, it is anticipated that the peptide acts very locally. This implies that somatostatin-producing cells must be in the vicinity of the target cells.

The bone marrow contains nerve terminals that produce multiple neuropeptides, including somatostatin (44–46). It has been suggested that nerve fibres contribute to the regulation of blood cell production and the release of blood cells from the marrow into the circulation (47, 48), although there is some controversy over these findings (49, 50). Nevertheless, it is possible that localized production of somatostatin by neural cells in the bone marrow contributes to homing of haematopoietic progenitors. An alternative, not mutually exclusive, possibility is that somatostatin is produced by the bone marrow stroma cells. Indeed, it is already known that stromal cells produce SDF-1 and SCF (16, 51). Somatostatin-producing cells were detected at the interface between bone and bone marrow, in close contact with vessels (52). At present we do not know what is the relevance of local production of somatostatin for the homing of haematopoietic progenitors *in vivo*. It also remains to be determined whether bone marrow stroma cells are capable of producing somatostatin. To date, we have been unable to detect somatostatin mRNA transcipts in a murine stromal cell line (FBMD-1). However, stroma is composed of several cell types; to determine whether bone marrow stroma cells can produce somatostatin, more stroma-derived cell lines and primary bone marrow stroma cultures have to be examined. Finally, we have examined whether somatostatin might act as an autocrine or paracrine regulator (or both) of migratory responses of bone marrow cells. We have consistently been unable to detect somatostatin mRNA in normal bone marrow and AML cells by RT-PCR, indicating that autocrine/paracrine activation of SST_2 does not have a role in migration of haematopoietic cells (S P M A Oomen, unpublished observations).

Conclusion and future perspectives

Our *in vitro* data have established that somatostatin, apart from inhibiting proliferation, exerts unique migration-inducing effects on normal and leukaemic haematopoietic progenitors. Somatostatin-induced migration may play a part in the homing and trafficking of these cells to different organs, and specific niches herein, during normal development and in pathological conditions. Therefore, a major challenge is now to establish the significance of these findings for haematopoietic stem cell migration *in vivo*.

Acknowledgements

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