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Review

SDF-1 and CXCR4 in normal and malignant hematopoiesis

J. Juarez and L. Bendall

Westmead Institute for Cancer Research, Westmead Millennium Institute, University of Sydney, Westmead, NSW, Australia

Summary. Over recent years it has become apparent that the chemokine SDF-1 and its receptor CXCR4 play pivotal roles in normal hematopoiesis. They are essential for the normal ontogeny of hematopoiesis during embryogenesis and continue to play a key role in retaining hematopoietic progenitors within the bone marrow microenvironment in the adult. As a result of this role disruption of SDF-1/CXCR4 interactions results in mobilization of hematopoietic progenitors and standard mobilization protocols disrupt this axis. Similarly SDF-1/CXCR4 interactions are required for homing and engraftment of hematopoietic stem cells during transplantation. SDF-1 regulates the localisation of leukemic cells and like their normal counterparts most leukemic cells respond to SDF-1 with increased adhesion, survival and proliferation. However in some instances leukemic cell responses to SDF-1 can be disregulated, the impact of which on the progression of disease in not known. In this review we discuss the pleiotropic roles of SDF-1/CXCR4 interactions in human hematopoietic stem cell ontogeny, bone marrow homing and engraftment, mobilization and how these interactions impact on malignant hematopoiesis.

Key words: SDF-1, CXCR4, Leukemia, Hematopoiesis,

Normal hematopoiesis

Hematopoiesis is a complex process whereby hematopoietic stem cells replicate and differentiate to produce all hematopoietic cell lineages. Hematopoietic stem cells are rare representing only 1 in 10,000 to 100,000 of total hematopoietic cells (Bonnet, 2002). They are characterised primarily by their ability to repopulate the entire hematopoietic system in conditioned transplant recipients. Although numerous surrogate markers exist for the identification of hematopoietic stem cells only a fraction of these cells are actual stem cells. Potential hematopoietic stem cells can be identified through expression of the cell surface antigen CD34 in humans or Sca-1 in mice and the absence of CD38 and lineage markers (Bonnet, 2002). The majority of these cells are quiescent and so can be defined by low DNA content using Hoechst staining and minimal metabolic activity with low staining for pyronin and rhodamine (Steinman, 2002). Hematopoietic stem cells initially give rise to lineage committed progenitor cells that can undergo further replication before they terminally differentiate into mature blood cells (Gunsilius et al., 2001).

In the foetus hematopoiesis occurs mainly in the liver, while in adults the principal hematopoietic organ is the bone marrow. The orderly proliferation and development of progenitor cells relies on the bone marrow microenvironment. The bone marrow microenvironment contains numerous cells of mesenchymal origin such as endothelial cells, fibroblast, adipocytes, osteoblasts, and cells of non-mesenchymal origin including macrophages (Waller et al., 1995; Gronthos and Simmons, 1996; Prosper and Verfaillie, 2001). These stromal cells produce and deposit a complex extracellular matrix (ECM), which includes fibronectin, laminin, collagen, hemonectin, thrombospondin, and proteoglycans (Zuckerman and Wicha, 1983; Wight et al., 1986). Stromal cells also produce cytokines and chemokines that can inhibit or induce hematopoietic progenitor cell proliferation and differentiation. In addition, cytokines and chemokines can be concentrated in particular niches within the bone marrow by varying local production and the presence of cytokine binding glycosaminoglycans (Netelenbos et al., 2003). Cytokines and adhesive ligands, present on stromal cells or within the ECM, are believed to influence the fate of hematopoietic progenitors (Prosper and Verfaillie, 2001). This is reflected in the differential localization of hematopoietic cells of distinct differentiation stages and lineage throughout different areas of the bone marrow space. Cells with immature morphology can be found close to the endosteal region adjacent to osteoblasts while more differentiated progenitors of the myeloid, erythroid and

Offprint requests to: Dr. Linda Bendall, Westmead Institute for Cancer Research, Westmead Millennium Institute, Westmead, NSW, 2145, Australia. Fax: 61-2-98459102. e-mail: linda.bendall@wmi.usyd.edu.au

megakaryocytic lineage can be found throughout the marrow (Islam et al., 1990; Nilsson et al., 1997; Prosper and Verfaillie, 2001). More recently, chemokines or chemotactic cytokines in particular stroma derived factor 1 (SDF-1) have also been shown to play a crucial role in the development of the hematopoietic compartment as well as in the regulation of hematopoiesis.

SDF-1 and CXCR4

SDF-1 is a CXC chemokine, which is broadly expressed and acts as a potent chemoattractant for immature and mature hematopoietic cells. It plays an important role in the homing of hematopoietic stem cells to the bone marrow and mediates the survival as well as the proliferation of human and murine colony-forming progenitor cells (Lataillade et al., 2000; Broxmeyer et al., 2003a). SDF-1 was first cloned from a bone marrow-derived stromal cell line by Tashiro et al. (1993) and was later identified as a pre-B-cell growth stimulating factor (PBSF) by Nagasawa et al. (1994). SDF-1 has two main isoforms: SDF-1α, the predominant isoform, consists of 68 amino acids; and SDF-1ß, which differs by an additional 4 amino acids (RLKM) at the C-terminal end due to alternative splicing (Shirozu et al., 1995). SDF-1 is thought to be the primordial chemokine due to its homology with both CXC and CC chemokine families (Shirozu et al., 1995; Bleul et al., 1996) and its high conservation across species with human and murine SDF-1 differing in only an Ile to Val substitution at position 18 (Shirozu et al., 1995). SDF-1 structure is well defined except for the N and C-terminal residues, amino acids 1-8 and 66-67 respectively. It adopts a typical chemokine fold consisting of three anti-parallel ß-strands and an overlying α-helix. Functional studies have shown that the first eight N-terminal residues of SDF-1 play an important role in both binding and activation of its receptor CXCR4, with the first two amino acids, Lys^1 and Pro^2 directly involved in receptor activation. The RFFESH motif (residues 12-17) in the well defined loop region of SDF-1, directly adjacent the second cystein, is essential for optimal binding but not sufficient for activation of CXCR4 (Crump et al., 1997). A two site model for SDF-1 receptor interaction has been proposed by Crump et al. (1997), which involves the initial docking of SDF-1 to CXCR4 through the RFFESH motif and subsequently activation of CXCR4 through the N-terminal residues of SDF-1. Pharmacological evidence for this mode of interaction has recently been shown with the bicyclam, AMD3100. AMD3100 specifically inhibits SDF-1 by blocking the binding of the N-terminal region to CXCR4 but does not affect the binding of the SDF-1 RFFESH domain to the SDF-1 N-terminus of CXCR4. Hence 3000 fold more AMD3100 is required to displace SDF-1 binding to CXCR4 than is required to block SDF-1 mediated responses such as chemotaxis (Gupta et al., 2001).

SDF-1 mediates its functions through its sole receptor CXCR4, a 352 amino acid, seven transmembrane G-coupled receptor protein which was originally identified as a coreceptor for T cell line tropic strains of HIV (Loetscher et al., 1994; Nagasawa et al., 1996b; Jazin et al., 1997). CXCR4 is highly conserved across species and is expressed on wide a variety of cell types including hematopoietic cells, vascular endothelial cells, neurons, microglia and astrocytes (Bleul et al., 1997; Hesselgesser et al., 1997; Gupta et al., 1998; Ohtani et al., 1998; Bajetto et al., 1999; Horuk, 2001). Several studies have shown that the N-terminal domain of CXCR4 is essential for potent binding of SDF-1, whilst the extracellular loop two which contains an acidic EADD motif plays an important role in receptor activation (Crump et al., 1997; Doranz et al., 1999; Brelot et al., 2000; Palladino et al., 2003). Zhou et al. (2001) have shown that the third extracellular loop of CXCR4 also plays a significant role in ligand binding and signalling. SDF-1 and CXCR4 have been implicated in numerous biological functions including cardiogenesis, neuronal development, vascular development in the gut and hematopoieisis as the result of studies on knockout mice (Nagasawa et al., 1996a; Ma et al., 1998; Tachibana et al., 1998). Here we will discuss what is known about the role of SDF-1 and CXCR4 in normal and malignant hematopoiesis.

SDF-1 and CXCR4 interactions in hematopoiesis

CXCR4 is expressed on the majority of hematopoietic cells including a significant proportion of the early $CD34^+CD38^-lin^-$ progenitors cells from bone marrow, cord blood, fetal liver and from mobilised peripheral blood (Aiuti et al., 1999a,b). Similarly a significant fraction of committed progenitors of myeloid, erythroid but particularly those of lymphoid lineages express CXCR4. Committed cycling progenitors also express SDF-1, possibly providing autocrine or paracrine stimulation of CXCR4 in hematopoietic cell cultures or in hematopoietic organs (Aiuti et al., 1999b; Lataillade et al., 2002). CXCR4 positive progenitors are responsive to SDF-1, which induces chemotaxis and stimulates integrin mediated adhesion of these cells (Aiuti et al., 1997; Peled et al., 1999a, 2000). Interestingly $CD34^+CXCR4^-$ cells contain intracellular CXCR4 and surface expression and the SDF-1 responsiveness of these cells can be induced by cytokines such as IL-6 and SCF (Kollet et al., 2002). Cord blood $CD34^+CD38^-lin^-CXCR4^+$ progenitors contain greater numbers of myeloid progenitors and fewer erythroid progenitors (Rosu-Myles et al., 2000). The reduction in erythroid colonies could be due to SDF-1 mediated suppression of human erythroid development (Gibellini et al., 2000). With the exception of committed erythroid progenitors, SDF-1 has survival-enhancing effects for hematopoietic progenitors including early T-lymphoid cells (Hernandez-Lopez et al., 2002), $CD34^+$ myeloid progenitors in steadystate human peripheral blood (Lataillade et al., 2002) and cord blood (Lee et al., 2002), and myeloid progenitors in

human (Broxmeyer et al., 2003a) and mouse bone marrow (Hodohara et al., 2000; Broxmeyer et al., 2003a). Although this effect is a direct effect on myeloid progenitors it could require synergistic actions of other cytokines (Lee et al., 2002; Broxmeyer et al., 2003a,b). When stressed $CD34^+$ cells upregulate CXCR4 expression and when exposed to SDF-1 decrease the apoptosis inducing Bad and Bax proteins and increase expression of Bcl-2. This is consistent with the survival promoting properties of this chemokine (Lataillade et al., 2002).

The effect of SDF-1 on the proliferation of hematopoietic stem cells is still uncertain. It is quite clear that SDF-1 has no significant mitogenic effect in vitro, however it does induce a G0 to G1 progression (Lataillade et al., 2002). This is likely to contribute to the synergistic effects on the proliferation of $CD34^+$ progenitors observed with Tpo and SCF. However it is possible that this is due to the proliferation of more mature committed progenitors (Lataillade et al., 2000, 2002). In stroma-dependent culture systems, the addition of exogenous SDF-1 inhibits cycling of early progenitors. This is consistent with data showing inhibition of stem cell cycling in vivo in response to high doses of SDF-1 (Cashman et al., 2002). It is possible that these conflicting data can be explained by opposing dose dependent effects of SDF-1 but it is more likely that the difference is due to the presence of stromal components. Integrin-mediated adhesion of hematopoietic progenitors to bone marrow stroma inhibits proliferation (Hurley et al., 1995; Verfaillie and Catanzaro, 1996). It is therefore likely that SDF-1 induced integrin mediated adhesion to stroma results in the observed inhibition of proliferation. There is also the possibility of synergistic effects with undefined stroma-derived factors including MIP-1α or TGF-ß (Bonnet et al., 1995; Batard et al., 2000).

The role of SDF-1 in the orchestration of hematopoietic development can be inferred from its pattern of expression in the developing embryo. The compartmental switch from the fetal liver to bone marrow correlates with the temporal and spatial expression of SDF-1 (McGrath et al., 1999). In postnatal life SDF-1 is produced in the bone marrow microenvironment, with highest levels being detected on osteoblasts and bone marrow endothelial cells (Lapidot, 2001). High levels of SDF-1 near the endosteum is of interest since early hematopoietic stem cells localize to this region of the bone marrow (Lambertsen and Weiss, 1984; Nilsson et al., 1997, 2001). The crucial role of SDF-1 production by the bone marrow microenvironment is apparent from the absence of bone marrow myelopoiesis and B lymphopoiesis in SDF-1 knockout mice and the successful hematopoietic reconstitution of wild type recipients transplanted with fetal liver cells from SDF-1 knockout mice (Nagasawa et al., 1996a; Ma et al., 1998). It appears that the main role of SDF-1 in myelopoiesis is the migration of progenitors from the fetal liver to the bone marrow during late embryogenesis. It is also required for the subsequent retention of progenitors within the bone marrow microenvironment with wild type mice transplanted with CXCR4 deficient cells (CXCR4 knockout chimeras), showing reduced granulocytic cells in the bone marrow and a 2-3-fold increase of granulocytes in the peripheral blood (Kawabata et al., 1999). Interestingly, the need for SDF-1 and CXCR4 by very primitive $c\text{-}kit^+Sca\text{-}1^+linlow/^-$ progenitors is less clear with normal numbers of progenitor cells being observed in the absence of CXCR4. The more committed $c\text{-}kit^+Sca\text{-}1\text{-}linlow/\text{-}$ cells are significantly reduced suggesting that signaling through CXCR4 is essential for either the transition of primitive cells to more committed progenitors or the successful expansion of these cells (Kawabata et al., 1999).

The absence of CXCR4 has profound negative effects on the pro-B, pre-B and immature B cell donor subsets within the bone marrow of the mice transplanted with fetal liver cells from CXCR4 knockout mice, with the B cell compartment displaying the greatest reduction in numbers of all donor cell lineages. This is in keeping with the requirement for SDF-1 and CXCR4 for the expansion and development of the earliest B cell precursors ($lin\text{-}CD19^-c\text{-}kit^+IL\text{-}7R\alpha^+AA4.1^+$) in mice (Egawa et al., 2001). In vitro, SDF-1 acts synergistically with other cytokines such as IL-7 to enhance the proliferation of precursor B cells (Nagasawa et al., 1994). The absolute requirement for CXCR4 was called into question by the presence of small numbers of B lymphoid progenitors at various differentiation stages in CXCR4 knockout chimeras (Kawabata et al., 1999; Ma et al., 1999). This may be due to the transfer of functional CXCR4 from wild type cells by microparticles during the transplantation procedure (Janowska-Wieczorek et al., 2001; Baj-Krzyworzeka et al., 2002). Examination of the CXCR4 status on cells after transplant will be required to determine the contribution of microparticles to the proliferation and differentiation of these cells. In stark contrast to B cell progenitors obtained directly from CXCR4 knockout animals, cell recovered from CXCR4 knockout chimeras proliferate and differentiate in Whitlock-Witte cultures at levels equivalent to those of wild-type progenitors (Ma et al., 1998, 1999; Zou et al., 1998). This suggests that CXCR4 expression is only required for a very narrow period of time during B cell ontogeny. Since the ability of $CXCR4^-$ stem cells from chimeric mice to undergo B lymphopoiesis has not been examined this remains speculation.

SDF-1 and CXCR4 do not appear to be essential for the production of mature B cells since CXCR4 knockout chimeras have small numbers of mature donor B cells repopulating their lymphnodes and spleens (Kawabata et al., 1999; Ma et al., 1999). This is consistent with the decline in SDF-1 responsiveness associated with B cell maturation (Fedyk et al., 1999; Honczarenko et al., 1999; Glodek et al., 2003). It is likely that diminished responses to SDF-1, including diminished adhesion to VCAM-1 and chemo-attraction, facilitate the egress of

maturing B cells from the bone marrow (Fedyk et al., 1999). However reduced numbers of mature B cells derived from $CD34^+$ progenitors expressing SDF-1 intrakine which prevents surface expression of CXCR4 mean that a role in mature B lymphopoiesis cannot be conclusively eliminated (Onai et al., 2000).

The role of SDF-1 and CXCR4 in the development of T lymphoid compartment is controversial. Thymic progenitors express high levels of CXCR4 and SDF-1 mRNA is highly expressed in the thymic subcapsular region, where thymic progenitors are located (Zaitseva et al., 1998; Suzuki et al., 1999; Hernandez-Lopez et al., 2002). SDF-1 supplementation leads to increases in thymocyte numbers in vitro and the absence of SDF-1/CXCR4 signaling results in inhibition of thymocyte proliferation and differentiation with an arrest occurring at the transition from $CD34^+$ precursur cells to $CD4^+$ immature thymocytes (Hernandez-Lopez et al., 2002). Similar to B lymphopoiesis, SDF-1 synergizes with IL-7 to enhance the expansion of thymic precursor cells and increases the viability of T cell precursors through increasing Bcl-2 and decreasing Bax protein levels (Hernandez-Lopez et al., 2002). Despite this in vitro evidence for a role for SDF-1 in T lymphopoiesis, results from in vivo studies have been conflicting. Normal T cell and thymic development in SDF-1 and CXCR4 knockout mice and one study using CXCR4 knockout chimeras suggest that SDF-1 and CXCR4 are not essential for T lymphopoiesis (Nagasawa et al., 1996a; Ma et al., 1998). However others using the same CXCR4 knockout and chimeric models showed a decrease in immature T cell subsets (Ara et al., 2003) and intrakine experiments revealed a partial arrest of thymocyte maturation from the double negative to the double positive state. This is more consistent with the in vitro data (Onai et al., 2000). Although the role of SDF-1 remains to be clarified there is substantial evidence for a role for this chemokine in normal T lymphopoiesis.

SDF-1 and CXCR4 in homing and engraftment

Although stem cell transplantation has been performed for over 30 years the mechanisms behind the homing of hematopoietic stem cells to the bone marrow is only now becoming elucidated. Hematopoietic stem/progenitor cell homing to the bone marrow requires concerted action between factors essential for cellular migration such as adhesion molecules and their ligands as well as chemotactic factors responsible for directing the lodgement of these cells in the correct microenvironment. This is believed to be a multistep process similar to the extravasation of leukocytes at sites of inflammation which involves rolling of cells along vessel walls, rapid activation of integrin function leading to tight adhesion and finally transendothelial cell migration (Butcher, 1991; Mackay, 1993; Bargatze et al., 1995; Springer, 1995; Butcher and Picker, 1996) Unlike most vascular endothelium, that in the bone marrow constitutively expresses E and P selectins, ICAM-1 and VCAM-1 (Candal et al., 1996; Schweitzer et al., 1996). These act as ligands for E and P-selectin ligands, and the integrins LFA-1, and VLA-4 respectively on transplanted hematopoietic progenitors facilitating their recruitment to the bone marrow (Mazo et al., 1998).

SDF-1 is a potent chemoattractant for hematopoietic stem cells (Aiuti et al., 1997) and activates numerous adhesion molecules essential to homing and engraftment such as VLA-4, VLA-5 and LFA-1 (Aiuti et al., 1997; Mohle et al., 1998; Imai et al., 1999; Peled et al., 1999a; Voermans et al., 2000; Hidalgo et al., 2001). In addition SDF-1 augments the production of the matrix metalloproteinases MMP-2 and MMP-9 by $CD34^+$ progenitors (Janowska-Wieczorek et al., 2000). These enzymes have been implicated in the invasion of tissue but their involvement in the homing of hematopoietic progenitors to the bone marrow is not known. It is likely that upon contact with SDF-1 displayed on bone marrow endothelium, that the integrins LFA-1 and VLA-4 on progenitor cells become activated leading to firm adhesion of the cell to the vessel wall. Cells then extravasate into the bone marrow extracellular compartment using LFA-1, VLA-4 and VLA-5 and migrate through the bone marrow toward local SDF-1 gradients (Teixido et al., 1992; Voermans et al., 2000). The necessity for SDF-1 and CXCR4 in stem cell homing to the bone marrow during late embryogenesis was demonstrated by knockout mouse studies. However, perhaps the most convincing evidence for the role of SDF-1 and CXCR4 in the homing and engraftment in the adult came from Peled et al. (1999b), who showed inhibition of human hematopoietic stem cell engraftment in NOD/SCID mice using neutralising antibodies to CXCR4 and CXCR4 downregulation. Homing and engraftment studies using sorted $CXCR4^+$ and $CXCR4^-$ populations have been hampered by the blocking of SDF-1 binding to CXCR4 by CXCR4 antibodies used in the sorting process (Rosu-Myles et al., 2000; Kollet et al., 2002). However, $CD34^+CD38^-$/lowCXCR4$^+$ stem cells selected by their chemotactic response to SDF-1, have a greater homing capacity than $CD34^+CD38^-$/lowCXCR4$^-$/low cells (Kollet et al., 2002). In addition, transplant patients who receive $CD34^+$ cells displaying high migratory capacity to SDF-1 show faster hematologic recovery (Voermans et al., 2001). Together, these studies strongly implicate SDF-1 as the primary chemotactic factor involved in the homing of hematopoietic progenitor cells to the bone marrow. It should be noted that $CD34^+CD38^-$/lowCXCR4$^-$ cells still display some homing capacity. The ability of CXCR4 antibodies to block homing of $CD34^+CD38^-$/lowCXCR4$^-$ progenitor fractions suggests that intracellular CXCR4 is mobilised to the cell surface and that this contributes to the homing of $CXCR4^-$ progenitors (Kollet et al., 2002). These data also raise concerns about the use of the NOD/SCID repopulating assay as a measure of "hematopoietic stemness" (ie the ability to repopulate all hematopoietic lineages). Intrafemoral injections have demonstrated stem cell

properties in cells that fail to engraft animals when administered by intravenous injection (Wang et al., 2003). Therefore it is important not to confuse engraftment potential with hematopoietic stem cell potential and the failure of cells to engraft following tail vein injection does not mean they lack stem cell potential.

Although the basic framework for the regulation of the movement of hematopoietic progenitors to the bone marrow has been determined, the interplay between factors such as SDF-1, adhesion molecules and growth factors that contribute to successful homing and engraftment of the bone marrow still need to be better defined. Homing of hematopoietic progenitors to the bone marrow is essential for successful hematopoietic stem cell transplantation. In this setting, the stem cell recipient has undergone some degree of exposure to DNA damaging agents such as ionising radiation and/or chemotherapeutic agents that enhance the expression of SDF-1 in the bone marrow (Ponomaryov et al., 2000). It is believed that up regulation of SDF-1 may be a protective mechanism to ensure the survival of hematopoietic stem cells and may also facilitate the homing of stem cells back to the bone marrow. The manipulation of CXCR4 expression on progenitors and SDF-1 expression in the bone marrow may help enhance engraftment efficiencies and could be used in the future to aid engraftment in transplant recipients.

SDF-1/CXCR4 and mobilisation

Under steady state conditions hematopoietic stem and progenitor cells are found primarily in the bone marrow although rare non-cycling stem and progenitor cells may also be found in the peripheral blood. Although the physiological role of steady-state peripheral blood stem cells is uncertain, it is likely they provide a mechanism for repopulating regions of damaged bone marrow (Wright et al., 2001). The release of greater numbers of hematopoietic progenitor cells into the periphery can be induced by a large number of agents including cytokines (G-CSF, GM-CSF, SCF, flt-3 ligand, IL-3, IL-7 and IL-12), chemokines (Mip-1α, Groß, IL-8 and SDF-1), sulfated glycans (fucoidan sulfate and dextran sulfate) and chemotherapeutic agents such as cyclophosphamide (Lapidot and Petit, 2002). This process, termed mobilisation was first described in the late 70s and is now widely used for the purposes of stem cell transplantation (To et al., 1997). G-CSF, alone or in combination with chemotherapy, is the principal mobilising agent used in the 30,000 transplants performed each year worldwide. Despite the common use of stem cell mobilisation, the mechanism behind the process is only now becoming understood, largely through our greater understanding of the role SDF-1 and its interaction with adhesion molecules in retaining stem cells in the bone marrow microenvironment. Occasionally patients or normal donors may mobilize poorly making an understanding the of underlying mechanisms important for improved clinical outcomes (Fu and Liesveld, 2000).

A role for SDF-1 in the retention of hematopoietic progenitors in the bone marrow has already been discussed and the presence of elevated progenitor numbers in the peripheral blood of CXCR4 knockout chimeras suggests that loss of bone marrow SDF-1 is sufficient for mobilisation (Ma et al., 1999). This is further supported by the mobilisation observed as a result of sulfated glycan-mediated release of SDF-1 from the bone marrow into the peripheral blood and reversal of the SDF-1 gradient between the bone marrow and peripheral blood by direct elevation of plasma levels of SDF-1 (Hattori et al., 2001; Sweeney et al., 2002). During clinical mobilisation protocols using G-CSF and/or cyclophosphamide, sharp increases in granulocytic precursors and mature granulocytes are seen within the bone marrow. This is accompanied by an abundant release of active neutrophil proteases including neutrophil elastase, cathepsin G and the matrix metalloproteinase, MMP-9 (Levesque et al., 2002). These proteases can cleave a number of molecules that have been implicated in the retention of hematopoietic progenitors within the bone marrow including the adhesion molecule, VCAM-1, the cytokine receptor, c-kit and both the chemokine, SDF-1 and its receptor, CXCR4 (Levesque et al., 2001, 2002, 2003a,b; McQuibban et al., 2001). Cathepsin G and neutrophil elastase also specifically cleave the N-terminal region of CXCR4 on hematopoietic progenitors resulting in loss of their chemotactic response to SDF-1 without loss of expression of the receptor as detected by most CXCR4 monoclonal antibodies (Valenzuela-Fernandez et al., 2002). This may explain data suggesting that CXCR4 is upregulated on progenitors following G-CSF treatment (Petit et al., 2002). The proteases that are most important in the mobilisation process is not clear but significant roles for neutrophil elastase, cathepsin G have been shown (Petit et al., 2002; Levesque et al., 2003a) while the role of MMP-9 is more controversial (Heissig et al., 2002). Elevated levels of SDF-1 expression by bone marrow stromal cells have been demonstrated following G-CSF administration. It is possible that this and the enhanced synthesis of CXCR4 by progenitors in the bone marrow in response to G-CSF may reflect an autocrine response to both decreasing concentrations of SDF-1 in the bone marrow and decreased signalling through CXCR4 due to proteolytic inactivation of this receptor by neutrophil enzymes.

Although loss of the SDF-1/CXCR4 axis within the bone marrow is sufficient to mobilize hematopoietic stem cells it is not the only interaction whose disruption is involved in this process. However preliminary data suggests that the addition of agents that antagonise SDF-1 binding to CXCR4 to current mobilization protocols further enhances the number of progenitors that can be recovered from the peripheral blood (Liles et al., 2002) making inhibition of SDF-1 interactions a potentially useful strategy from improving stem cell mobilization

protocols.

SDF-1 in leukemic hematopoiesis

Leukemias are malignant neoplasms of hematopoietic cells. Historically, they have been categorised based on morphological characteristics and named after the closest normal cell type. More recently cytogenetics is playing an increasing role in the classification of leukemias. Leukemic cells undergo uncontrolled expansion due to increased proliferation and/or reduced cell death. Despite this, leukemic cells retain many characteristic of the normal cells of similar lineage and maturation. In most instances CXCR4 expression is similar on normal and malignant cells of the same lineage and maturation stage (Mohle et al., 1998; Burger et al., 1999; Bradstock et al., 2000; Durig et al., 2000; Dialynas et al., 2001). The most striking exception is the significantly elevated CXCR4 levels detected on leukemic cells from patients with B chronic lymphocytic leukemia (B-CLL). Accordingly B-CLL cells are more responsive to SDF-1 in trans-migration assays than their normal counterparts (Mohle et al., 1999).

Blasts from patients with acute myeloid leukemia (AML) demonstrate variable CXCR4 expression which is correlated to SDF-1 driven trans-endothelial migration (Mohle et al., 1998). Correlation between CXCR4 expression and AML subtypes is however less clear with one study finding association between high expression levels and AML samples of well differentiated myelomonocytic phenotype and another with immature phenotype, a finding paralleling normal progenitors (Mohle et al., 2000; Voermans et al., 2002). In keeping with observations in normal myeloid progenitors, AML blasts isolated from the peripheral blood have reduced SDF-1 responses compared to those isolated from the bone marrow of the same patient. A role for CXCR4 and SDF-1 in retaining AML cells in the bone is further supported by the lower blast numbers in the peripheral blood of patients whose leukemic cells showed strong responses to SDF-1 (Voermans et al., 2002). In contrast blasts from patients with chronic myeloid leukemia (CML) have attenuated migratory and adhesive responses to SDF-1 although levels of CXCR4 expression are normal (Salgia et al., 1999; Peled et al., 2002). Like normal progenitors, CML cells isolated from peripheral blood display reduced responses to SDF-1 compared to bone marrow derived leukemic cells. CML cells also display normal calcium fluxes and internalisation of CXCR4 following exposure to SDF-1 suggesting that the defect is downstream of these early events (Durig et al., 2000). The BCR/ABL proto-oncogene is responsible for the leukemic phenotype in CML by interfering with intracellular signalling pathways that regulate cell proliferation and survival. BCR/ABL also seems to be responsible for the attenuated responses of CML blasts to SDF-1 (Salgia et al., 1999). A distinctive feature of CML blasts is their reduced integrin mediated adhesion to numerous substrates including bone marrow stromal layers (Bhatia et al., 1994). It is reasonable to assume that integrin disfunction in CML cells is largely responsible for the attenuation of SDF-1 induced adhesion and migration in these cells, although BCR/ABL may also directly interfere with SDF-1 induced signalling (Ptasznik et al., 2002).

The picture in acute lymphoblastic leukemias (ALL) is complex with leukemias of T cell origin being completely refractory to SDF-1 despite expressing CXCR4 (Dialynas et al., 2001). In contrast, most ALL cells with a B cell progenitor phenotype are highly responsive to SDF-1, with cells demonstrating SDF-1 induced calcium flux, integrin mediated adhesion, chemotaxis and migration (Bradstock et al., 2000; Shen et al., 2001). CXCR4 also mediates homing and engraftment of pre-B ALL cells to the bone marrow of NOD/SCID mice (Shen et al., 2001). However, unlike AML blasts there is no correlation between expression of CXCR4 and migratory or chemotactic responses to SDF-1 (Sbaa-Ketata et al., 2001). This could be partially explained by a mirroring of the decreased responsiveness to SDF-1 in the absence of an equivalent reduction in CXCR4 expression observed in maturing normal B cell progenitors. However, there are some cases of pre-B ALL that completely fail to chemotax in response to SDF-1 despite undergoing calcium flux and CXCR4 internalisation reminiscent of CML regardless of maturation. The reason for the lack of chemotactic responses in these cases is not known.

SDF-1 has been reported to enhance the survival and proliferation of leukemic cells. Probably the best documented of these is the enhanced survival of B-CLL cells resulting from contact with “nurse cells” which are derived from peripheral blood myeloid cells. Enhanced leukemic cell survival is due to inhibition of apoptosis and is partially mediated by SDF-1 produced by these cells (Burger et al., 2000; Burger and Kipps, 2002). The situation is less clear in pre-B ALL. Inhibition of apoptosis associated with increased Bcl-2 expression was observed using SDF-1 containing conditioned medium and neutralising SDF-1 antibodies (Nishii et al., 1999). However, experiments using stroma supported cultures with SDF-1 antagonist, and the direct measurement of the effects of SDF-1on pre-B ALL survival both alone and in combination with other cytokines in serum free conditions failed to reveal any effect on pre-B ALL cell survival (Juarez et al., 2003). In contrast we did observe an inhibition of stromal dependent proliferation of pre-B ALL cells in the presence of SDF-1 antagonists including AMD3100 and polyphemusin II derived peptides. In addition we have observed synergistic effects of SDF-1 on pre-B ALL cell proliferation when combined with IL-3 and IL-7 in serum free cultures (unpublished observations).

Considering the important role played by SDF-1 in normal hematopoiesis it is possible that CXCR4 mediated signals could play a role in leukemogenesis. It

is likely that CXCR4 and SDF-1 play a major role in the spread of leukemias from one marrow site to another. In support of this elevated CXCR4 expression has been implicated in bone marrow invasion by B-CLL cells and has recently been associated a poor outcome in these patients (Ishibe et al., 2002). In contrast, invasion of lymph nodes by these cells appears to be independent of CXCR4, relying on other chemokine receptors (Till et al., 2002). Similarly high CXCR4 expression has been implicated in the development of extramedullary medullary relapse in pre-B ALL (Crazzolara et al., 2001) although others have not found this association (Schneider et al., 2002). There is evidence to suggest that high CXCR4 expression may be prognostic for pre-B ALL (Schneider et al., 2002) but a larger study is required to confirm this. In contrast, there is no evidence for the prognostic value of CXCR4 in myeloid leukemias (Brouwer et al., 2001) although there is a sparsity of published data.

Antagonists of SDF-1 may prove to be useful therapeutic agents for the treatment of some leukemias. SDF-1 can antagonise the apoptotic effects of some drugs on B-CLL (Sarfati et al., 2003) and we have shown that antagonists of SDF-1 augment the effectiveness of vincristine and dexamethasone on pre-B ALL cells in stroma-dependent cultures in vitro (Juarez et al., 2003). SDF-1 antagonists are available for use in patients and are currently in clinical trial in other disease settings. SDF-1 antagonists are most likely to effective in the treatment of B cell leukemias where SDF-1 supports the proliferation and survival of the leukemic cells even in the presence of chemotherapeutic agents. However, further in vivo studies are required to assess the likely effectiveness of these agent in the treatment of B cell leukemias.

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