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Consequences of HIV infection in the bone marrow niche

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Dysregulation of the bone marrow niche resulting from the direct and indirect effects of HIV infection contributes to haematological abnormalities observed in HIV patients. The bone marrow niche is a complex, multicellular environment which functions primarily in the maintenance of haematopoietic stem/progenitor cells (HSPCs). These adult stem cells are responsible for replacing blood and immune cells over the course of a lifetime. Cells of the bone marrow niche support HSPCs and help to orchestrate the quiescence, self-renewal and differentiation of HSPCs through chemical and molecular signals and cell-cell interactions. This narrative review discusses the HIV-associated dysregulation of the bone marrow niche, as well as the susceptibility of HSPCs to infection by HIV.

KEYWORDS

bone marrow niche, HIV, haematopoiesis, haematopoietic stem/progenitor cell, dysregulation

Introduction

The existence of the bone marrow niche was first proposed by Schofield (1) as a specialised microenvironment for the maintenance of haematopoietic stem and progenitor cells (HSPCs), and can be found in the marrow of long bones, vertebrae and iliac crest. The presence of multiple niches within the bone marrow has been proposed due to the presence of distinct subsets of HSPCs in close proximity to non-haematopoietic cell types (2). The bone marrow niche consists of bone matrix and various non-haematopoietic cells, including endothelial cells, stromal cells, neuronal cells and adipocytes (3). Cells of the niche contribute directly to HSPC quiescence, tethering in the bone marrow, homing to niche regions and mobilisation into the circulation, as well as differentiation through intercellular contact and paracrine signalling (3–5).

The bone marrow niche is separated into endosteal (6–8) and perivascular regions (4, 5, 9), each thought to serve a distinct function in the maintenance and mobilisation of HSPCs. Osteolineage cells, perivascular mesenchymal stromal/stem cells (MSCs), CXC chemokine ligand (CXCL)12-abundant reticular (CAR) cells and endothelial cells produce chemoattracting gradients of CXCL12 (also known as stromal-derived factor 1 (SDF-1) and stem cell factor (SCF)) which draw HSPCs to both the endosteal and perivascular

regions (4, 5, 7). Non-myelinating Schwann cells and megakaryocytes activate transforming growth factor beta (TGF-β) which has been implicated in maintaining quiescence (5). Megakaryocytes are also thought to contribute to the niche function by releasing CXCL4 and small amounts of thrombopoietin (TPO) which encourage quiescence (4). Coupled with long-range and short-range cytokines regulating haematopoiesis, signalling networks in the bone marrow are extremely complex and have not been fully elucidated. The process of haematopoiesis is well studied and involves many cytokines, chemokines, cell-to-cell interactions and extracellular matrix interactions. However, the *in vivo* functionality, frequency and longevity of HSPCs in humans has not been fully defined.

The classical model of haematopoiesis is represented by a hierarchical structure with long-term HSPCs at the apex of the hierarchy (10–12). These cells possess self-renewal capabilities and give rise to short-term HSPCs with limited self-renewal capabilities. Short-term HSPCs differentiate to form multipotent progenitors (MPP), which are precursors of common lymphoid and myeloid progenitors (CLPs/CMPs). MPPs are not able to self-renew but are capable of full lineage differentiation (13). Progeny of CLPs differentiate into lymphoid and natural killer (NK) cells, while progeny of CMPs form granulocyte–macrophage progenitors (GMP) or megakaryocyte–erythrocyte progenitors (MEP). These differentiate into granulocytes and macrophages, and erythrocytes and megakaryocytes, respectively (14). Recent studies suggest that haematopoiesis is more complex than the classical model makes provision for, which includes myeloid-restricted progenitors with long-term repopulating potential (15) and HSPCs expressing platelet-biased genes while having the ability to self-renew (16). These examples represent only a fraction of the data demonstrating the non-classical differentiation potential of HSPCs. Technological advances in the past decade have resulted in a revised model depicted in Figure 1.

Since the discovery of the human immunodeficiency virus (HIV) in the early 1980s (17–20), it has spread globally, infecting

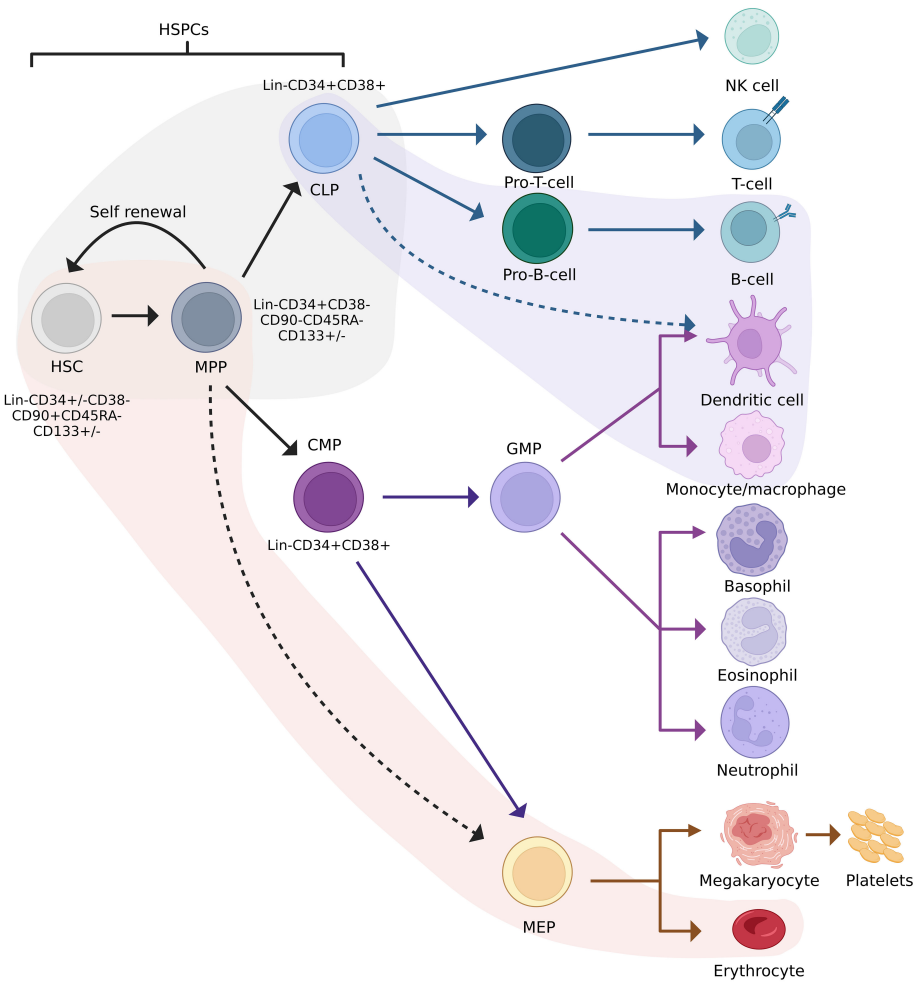

FIGURE 1
Revised model of haematopoiesis including HSPC phenotypes. Red and purple streams indicate lineage preferences. The grey area indicates cells between which lineage commitment may be reversed. HSC, haematopoietic stem cell; HSPC, haematopoietic stem/progenitor cell; MPP, multipotent progenitor; CLP, common lymphoid progenitor; CMP, common myeloid progenitor; GFM, granulocyte-macrophage progenitor; MEP, megakaryocyte-erythrocyte progenitor. Adapted from Velten et al., 2017, Brown et al., 2018, and Liggett & Sankaran, 2020. Figure created in BioRender.com.

more than 38 million people worldwide according to the latest available statistics (21). The ability of the virus to evade the immune system (22) and escape antiretroviral therapy (ART) pressure (23) contributes to its persistence *in vivo*. Drug escape and immune evasion are achieved through the action of viral proteins (24–26), immune dysregulation as an indirect consequence of infection (27–30), as well as the high mutation rate of the virus conferred by the low-fidelity HIV reverse transcriptase enzyme (31–33). The majority of HIV infections are caused by HIV-1 group M strains (34), with HIV-1 subtype B (HIV-1B) and C (HIV-1C) claiming 11% and 48% of worldwide infections, respectively (35). Most HIV-1 infections in India, southern Brazil (36) and sub-Saharan Africa are due to HIV-1C (37), while HIV-1B is confined to high income regions such as North America, Europe and Australia (37–40). Despite the prevalence of HIV-1C, HIV-1B dominates the research landscape. Variation between subtypes has been documented for phenotypic properties such as co-receptor tropism (41–45), replication rate and disease progression (45–50), transmission mechanics (51–54), and mutation patterns (55–57). Furthermore, reverse transcription (58) and the emergence of drug resistance (59–62) have been reported to vary between subtypes.

HIV primarily infects cells of the immune system that express cluster of differentiation (CD) 4, C-C-motif chemokine receptor type 5 (CCR5) and C-X-C-motif chemokine receptor type 4 (CXCR4), including CD4+ T-cells and monocyte/macrophages (63); in the case of the former, this results in the depletion of CD4+ cells. The viral reservoir is made up of latently-infected cells which harbour proviral DNA but do not produce viral particles (64). Once established, the reservoir is the most challenging barrier to curing HIV. Latency is complex and regulated at several levels, reviewed elsewhere (64–67). Activation of viral production from latently-infected cells contributes to viral persistence throughout the lifetime of an infected individual. This is evidenced through lineage-tracing which has shown the resurgence of sequences that were dominant during early infection, in the later stages of infection (68–74).

HIV has several concomitant effects once an individual becomes infected. Direct infection of cells with HIV is not the only cause of blood cell depletion, referred to as cytopenia, in HIV patients. Multifactorial, indirect effects associated with HIV infection can also cause cytopenia and other haematological abnormalities. A plethora of cytopenias may present in HIV patients including leukopenia, lymphopenia, anaemia, neutropenia, thrombocytopenia, and pancytopenia (10, 75–81). In a large study conducted in Beijing, neutropenia, thrombocytopenia, and anaemia were partially restored in ART-naïve patients following initiation of treatment (76). However, multiple factors influence restoration of cytopenia following induction of ART (82) including concomitant infections, viral load, tropism and drug resistance, and individual response/adherence to treatment. With the exception of lymphopenia, HIV-associated cytopenias cannot be explained by the lytic cycle of HIV infection. It is unclear whether the haematological abnormalities observed in HIV-infected individuals are due to direct or indirect effects of infection on HSPCs. Various studies suggest these cytopenias may be attributed to disruption of the bone marrow niche housing HSPCs, which maintain the continuous production of blood and immune cells throughout life (83–85). This review will discuss both these possibilities in detail.

Indirect effects of HIV on HSPCs

The bone marrow is considered a primary and secondary lymphoid organ allowing for continuous interactions of immune cells (86). The indirect effects of HIV on HSPCs may stem from infection of bone marrow niche cells (87), the effects of HIV proteins on bone marrow cells, or dysregulation of the cytokine milieu which is instrumental in orchestrating dynamic physiological processes including haematopoiesis. The effects of HIV infection on bone marrow niche cells and the consequences for haematopoiesis are described in detail below and are illustrated in Figure 2.

Perivascular niche

The perivascular region around blood vessels that permeate the bone marrow contains perivascular cells, endothelial cells, CAR cells, and nerve fibres including non-myelinated Schwann cells.

Non-myelinating Schwann cells sheath neuronal axons in the perivascular niche of the bone marrow, and participate in niche regulation (88). These cells have been found to activate latent TGF-β released from the bone marrow extracellular matrix (89), and to facilitate circadian regulation of CXCL12 production in Nestin+ MSCs (88). Together, these functions probably account for the maintenance of a quiescent perivascular HSPC pool very closely associated to neuronal axons. Non-myelinating Schwann cell depletion results in reduced HSPC numbers as early as three days post-depletion (89), although the mechanism is unclear. Investigation into HIV-associated neuropathy revealed that the HIV glycoprotein (gp)120 protein stimulated lysosomal exocytosis in Schwann cells (90), releasing axon-exciting adenosine triphosphate (ATP) into the extracellular environment. Exocytosis of lysosomes increased calcium and induced reactive oxygen species (ROS) generation in neighbouring axons, which in turn activates latent TGF-β (91). This could contribute to impaired haematopoiesis by driving HSPCs toward quiescence. Since infection of Schwann cells has only been documented once by electron microscopy (92), HIV-associated neurotoxicity is likely caused by either viral proteins or neurotoxic cytokines released by activated/infected glial cells. The interaction between gp120 and CXCR4 on Schwann cells results in the release of several chemokines, including CC chemokine ligand (CCL)-5 (also known as RANTES) and CXCL1. Release of CCL5 results in the production of TNF-α by dorsal root ganglion neurons and subsequent autocrine neurotoxicity mediated by TNFR1 (93), whereas the release of CXCL1 results in the recruitment of macrophages in mice (94).

Several subtypes of endothelial cells, the cells which line blood vessels, have been identified in the bone marrow, and the distinct functions of each subtype are still being elucidated. Arteriolar, sinusoidal, and endothelial cells expressing endoglin (CD105) are

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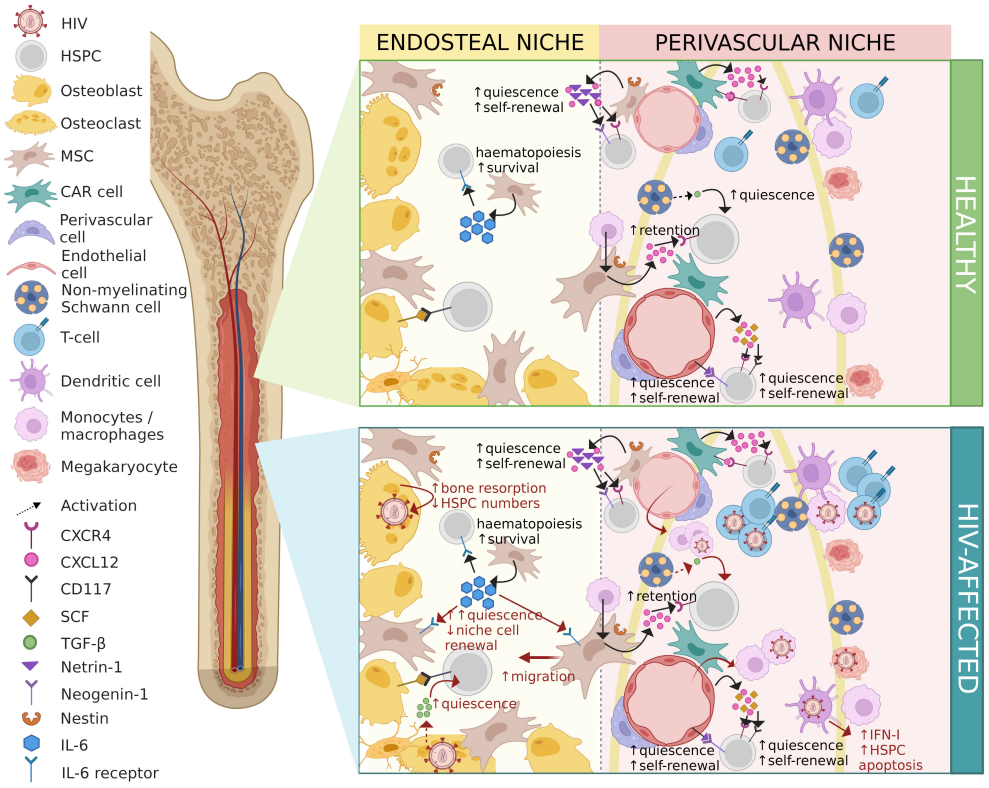

FIGURE 2
Contrasting healthy and HIV-affected bone marrow niches. HIV-associated changes are shown in red. HIV, human immunodeficiency virus; HSPC, haematopoietic stem/progenitor cell; MSC, mesenchymal stem/stromal cell; CAR cell, CXCL12-abundant reticular cell; CXCR4, C-X-C-motif chemokine receptor type 4; CXCL12, C-X-C-motif chemokine ligand 12; CD117, cluster of differentiation molecule 117; SCF, stem cell factor; TGF-β, transforming growth factor-beta; IL-6, interleukin-6; IFN-I, interferon type-I. Figure created in BioRender.com.

among these. Netrin-1 is expressed by arteriolar endothelial cells and binds to the receptor Neogenin-1 on HSPCs, which is correlated with quiescence and self-renewal of HSPCs *in vivo* (95). Furthermore, arteriolar endothelial cells have been found to produce the majority of endothelial cell-derived SCF, in addition to producing CXCL12. Knock-out of *SCF* in arteriolar endothelial cells results in reduced CD150+CD48-Lin-Sca-1+c-Kit+ primitive HSPCs in mice (96, 97). In bone marrow injury such as irradiation or chemotherapy, an endothelial cell population expressing endoglin (CD105) produces interleukin (IL)-33, which expands umbilical cord blood-derived CD34+ HSPCs *in vitro* and promotes angiogenesis and osteogenesis for bone marrow regeneration (98). Work on the cytokine profiles produced by different endothelial subtypes is lacking. Previous studies in the 1990s showed that endothelial cells produce messenger RNA (mRNA) for cytokines supporting and inhibiting haematopoiesis (99, 100). Cytokines supporting haematopoiesis include granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1, IL-7, IL-6, TGF-β, IL-8, and IL-11, while thymosin-β4 is a small molecule that inhibits haematopoiesis (100). Endothelial cells also produce mRNA for macrophage inflammatory protein (MIP)-2, platelet-derived growth factor (PDGF), merozoite surface protein (MSP)-1, interferon (IFN)-γ, IL-13 and inhibitin (100). However, the relationship between cytokine mRNA and protein production by endothelial cells for these cytokines is unclear.

In a study comparing bone marrow microvascular endothelial cells from HIV seropositive to those from healthy uninfected donors, these cells have been shown to be permissive to HIV infection *in vivo* (101). HIV-infected microvascular endothelial cells expressing von Willebrand Factor (vWF) were found to produce the HIV protein p24 in long-term culture. Endothelial cells from HIV seropositive donors showed a significant reduction in IL-6 and GM-CSF production in response to IL-1α stimulation compared to uninfected controls (101). In addition to being susceptible to productive HIV infection *in vivo*, HIV proteins contribute to endothelial cell activation, apoptosis, and conversely, stimulate angiogenesis, proliferation, and migration of endothelial cells through various mechanisms (102, 103).

The HIV trans-activator of transcription (Tat) protein induces apoptosis in endothelial cells, but also induces the release of IL-6 and the expression of adhesion markers E-selectin, intracellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, and endothelial leukocyte adhesion molecule (ELAM)-1 which recruit monocytes and increase their migration across the endothelial barrier, increasing monocyte tissue pervasion (104–106). The HIV negative factor (Nef) protein induces apoptosis in endothelial cells by increasing ROS production, causing oxidative stress and cell death (107–111) as well as increasing production of monocyte attractant protein (MCP)-1 (109). The HIV matrix protein p17, similar to Tat, promotes angiogenesis (112) and

increases monocyte chemoattractant protein (MCP)-1 production in endothelial cells, as does Nef (113, 114). The implications of these contrasting consequences of HIV infection on endothelial cells *in vivo* remain to be resolved.

CXCL12 is primarily produced by CAR cells (115, 116) and osteoblasts (117) in the bone marrow, and is involved in the homing of cells expressing the cell-surface marker CXCR4 (118, 119), thereby acting as a potent chemoattractant for HSPCs and their progeny in the bone marrow. In addition to chemoattraction, CXCL12-CXCR4 interactions provide physical tethering of CD34+ HSPCs to cell-surface CXCL12 on CAR cells (116, 120). The CXCR4-CXCL12 axis has been found to be critical for the maintenance of the primitive HSPC pool, which is diminished in CXCR4 knock-down mice (116). Neither direct nor indirect effects of HIV infection have been reported for CAR cells to date. There is ongoing debate as to whether a variant in the untranslated region of CXCL12 (designated 3'A) is protective against HIV infection and delays disease progression or whether it is associated with susceptibility to HIV infection and faster progression to AIDS (121).

Dendritic cells and macrophages are both present in the bone marrow stroma in the perivascular niche forming so-called "immune pockets", where B- and T-lymphocytes are localised. While bone marrow dendritic cells do not play an appreciable role in HSPC maintenance or haematopoiesis, selective ablation of dendritic cells results in increased HSPC mobilisation through an indirect mechanism involving CXCR2 (122). Dendritic cells and macrophages are capable of sustaining HIV replication and could contribute to viral dissemination in the niche (123–125). The perivascular niche is also home to megakaryocytes, which have been found to be susceptible to HIV infection both *in vitro* (126–128) and *in vivo* (128, 129). This relates primarily to viral production and release in the bone marrow and could contribute to thrombocytopenia in HIV patients through the loss of megakaryocytes as a consequence of viral replication.

Circulating monocytes are recruited to tissues and differentiate into tissue-resident macrophages, where they fulfil critical functions in tissue homeostasis. Most of the research on bone marrow macrophages to date has involved murine studies, although whether the findings translate to human bone marrow is not clear. Two populations of bone marrow macrophages have been identified in mice, so-called "osteomacs" present in the endosteal niche in close contact to osteoblasts and Nestin+ MSCs (130), and CD169+ macrophages located in the perivascular niche around Nestin+ MSCs (131). Osteomacs, through cell-to-cell contact, increase Nestin+ MSC production of prostaglandin (PG)-E_2, which in turn stimulates oncostatin M release by osteomacs, resulting in increased osteoblast mineralisation and differentiation (132). Higher levels of PGE_2 correlated with increased release of anti-inflammatory IL-10 (133, 134) by macrophages (135). Depletion of CD169+ bone marrow macrophages severely impaired HSPC retention in the niche, and this was associated with a reduction in Nestin+ MSC expression of CXCL12 and SCF mRNA (136, 137). Bone marrow macrophages have been reported to be permissive to HIV infection, although the cytokine profile did not appear to be altered *in vitro* following infection with a number of HIV isolates (138). Whether HIV infection occurs predominantly in monocytes prior to tissue specification into macrophages or in tissue-resident macrophages is difficult to establish. Macrophages may be an important tissue reservoir for HIV capable of sustaining HIV infection *in vivo* (87, 139–142) and their presence would therefore form a large part of the barrier to HIV eradication. Conversely, other researchers have suggested that macrophages play a limited role in HIV replication (143–145). While these cells can be infected by HIV, they may not effectively support viral replication and production. As a result, their contribution to viral spread and long-term persistence in the body may be minimal. Although drawing parallels between murine and human bone marrow is beyond the scope of this review, it is plausible that macrophage depletion resulting from direct HIV infection may result in reduced HSPC retention in the niche and thereby contribute to impaired haematopoiesis in humans.

Endosteal niche

The endosteal niche is in close proximity to the endosteum of the bone marrow niche which is made up of osteoclasts, osteoblasts, and MSCs. Osteolineage cells (osteoblasts and osteoclasts) were some of the first cells shown to interact with HSPCs and play an important role in HSPC fate. MSCs destined to become osteoblasts occupy the endosteal surface of flat and trabecular bones between the bone and the bone marrow.

Bone marrow MSCs, initially thought to be fibroblasts (146–148), are a heterogenous population of cells forming part of both the perivascular and endosteal stromal cell populations. In addition to replacing osteoblasts and adipocytes as a normal part of cell turnover, bone marrow MSCs play an important role in immunomodulation and HSPC maintenance through cytokine production (149). MSCs constitutively produce IL-6, which is important in haematopoiesis and suppressing the proliferation of MSCs and activated T-cells (149–151). MSCs have also been shown to secrete prostaglandin E_2 (PGE_2), which is implicated in the expansion of less primitive HSPCs (152) as well as HSPC recovery and repopulation after chemotherapy (153, 154). In the perivascular niche, periarteriolar MSCs produce Netrin-1 (95) similar to arteriolar endothelial cells, thereby contributing to HSPC quiescence and self-renewal. MSC heterogeneity in the bone marrow is much better described in mice than in humans, and this includes single-cell resolution as has been extensively reviewed elsewhere (155, 156). In murine bone marrow, Lepr+ MSCs enriched for adipocyte and osteoblastic precursors secreting SCF (157) and CXCL12 (116) have been described, which are implicated in HSPC self-renewal (158) and quiescence, respectively, have been described. Although LEPR(hi)CD45(low) BM-MSCs were recently identified in human marrow, the function of these cells remains poorly described (159). Nestin+ MSCs are typically periarteriolar and were found to be clustered around nerve fibres where they produce CXCL12 (156). Characterisation of human bone marrow MSC heterogeneity at the single-cell level remains crucial to elucidating the complex niche dynamics supporting HSPCs, haematopoiesis, as well as immunomodulation in the bone marrow.

While the susceptibility of bone marrow MSCs to HIV infection has not yet been conclusively determined (160), more studies suggest low levels of productive infection - referring to the production of new virus particles (161–163) than studies which demonstrate resistance to infection (138). However, exposure to HIV proteins has varying effects on the differentiation of MSCs. HIV Tat and Nef proteins reduce MSC proliferation and differentiation, and encourage senescence, corresponding to increased oxidative stress and mitochondrial dysfunction (164). Tat increases nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) activity and inflammatory cytokine secretion, while Nef reduces autophagy, and it was found that the effects of Tat and Nef are cumulative (164). NF-κB expression also drives IL-6 production in MSCs (149), resulting in increased MSC senescence which could explain dysregulation of both bone and fat metabolism in HIV patients. Regulator of expression of virion (Rev) and p55-gag protein expression results in temporal and quantitative changes in key osteo- and adipogenic signals, hampering differentiation (165). Expression of both Rev and p55-gag increase alkaline phosphatase activity and decrease lipid levels, where Rev increases calcium deposition in non-differentiating MSCs. Rev also increases potent peroxisome proliferator-activated receptor gamma (PPAR-γ) expression which drives adipogenic differentiation, and Runt-related transcription factor (RUNX)2 which drives osteogenic differentiation in non-differentiating MSCs (165). In a study assessing the effects of HIV proteins on human MSCs and osteoblast cell lines, HIV proteins p55 and gp120 reduced calcium deposition, alkaline phosphatase activity, and key bone remodelling proteins (166). In contrast, the HIV Rev protein augments MSC osteogenesis (166). HIV gp120 improves adipogenic differentiation, impairs endothelial differentiation, and induces apoptosis of vessel wall-derived MSCs (161). These findings support a role for MSCs in haematopoietic abnormalities resulting from HIV-associated bone marrow niche dysregulation.

Osteoclasts are large, multinucleated monocyte/macrophage-derived cells responsible for resorption of the bone matrix produced by osteoblasts in the continuous, dynamic process of bone remodelling (167, 168). Osteoblasts are smaller osteolineage cells derived from MSCs and produce macrophage colony-stimulating factor (M-CSF) for osteoclastogenesis, osteopontin for continuous formation of bone matrix, as well as a variety of bone marrow niche regulatory cytokines (169). These include IL-6, MIP-1α, SCF, CXCL12, granulocyte colony-stimulating factor (G-CSF), TPO, angiotensin-1, and annexin 2 (169). The bone matrix maintained by osteoclasts and osteoblasts results in a protected environment for long-term HSPCs. Bone remodelling results in the release and activation of TGF-β stored in the bone matrix (168), and a calcium gradient (170), both of which contribute to the quiescence of HSPCs in the endosteal niche. Immature osteoblasts release CXCL12, a potent chemoattractant for cells such as HSPCs which express CXCR4 (169), drawing them towards the endosteum where they bind to SCF on mature osteoblast cell surfaces through the CD117 receptor (169). Angiotensin-1 (171) and TPO (172) produced by osteoblasts also assist in maintaining HSPC quiescence in the endosteal niche.

In HIV infection, bone resorption is increased due to the stimulation of osteoclastogenesis by HIV proteins and direct infection of osteoclasts (173–175). This increased osteoclast activity has been associated with reduced HSPC numbers in the bone marrow (176). In addition to propagating virus through replication, osteoclasts have been implicated in cell-to-cell transmission of HIV-1 between cells of the bone marrow niche (177). Osteoblasts are reportedly not susceptible to HIV infection *in vitro* (178), but respond deleteriously to the presence of HIV proteins. Alkaline phosphatase activity, receptor activator of nuclear factor kappa-B ligand (RANKL) secretion, and calcium (Ca^{2+}) deposition by osteoblasts have been reported to be impaired in the presence of HIV p55-gag and gp120 proteins (179). Studies on osteoclasts have produced conflicting findings regarding the induction of apoptosis versus proliferation following exposure to HIV-1 gp120 (180, 181). Degradation of the bone matrix during bone resorption releases and activates an excess of TGF-β in the endosteal niche (182). In addition to stimulating and recruiting MSCs from the perivascular niche to the endosteal niche, high levels of active TGF-β induce quiescence in primitive HSPCs (91), promote proliferation and differentiation of myeloid-primed HSPCs, and hinder lymphoid-primed HSPCs (183). Bone resorption, reduced osteoblast activity in the presence of HIV proteins, and impaired differentiation of MSCs into osteoblasts all contribute to loss of bone density and overall osteopenia observed in HIV patients (184, 185). Consequently, reductions in IL-6, MIP-1α, SCF, CXCL12, G-CSF, and TPO usually produced by osteoblasts are expected in HIV patients. Bone disease in HIV-infected individuals suggests that some of the *in vitro* findings may be transferable (186), although anti-retroviral therapy (ART) has also been implicated (185–187).

Productive infection of bone marrow niche cells would result in the release of HIV proteins which consequently would have an adverse effect on the infected cell and surrounding cells which in turn would negatively affect HSPCs.

Infiltrating/circulating cells

The bone marrow is highly vascularised, allowing the trafficking of cells and chemical signals to and from the bone marrow through the circulation. In addition to localised effects of HIV on bone marrow cells, altered cytokine profiles produced by trafficked cells affect the niche microenvironment. Monocytes originating from the bone marrow enter the circulation and differentiate into macrophages or dendritic cells in tissues, where they may become infected with HIV (188). Upon infection and pathogen-associated molecular pattern (PAMP)/toll-like receptor (TLR)-initiated migration to secondary lymphoid tissues, dendritic cells are involved in cell-to-cell transfer of HIV to T-cells (189–191). In addition to perpetuating viral transmission, activation of PAMP triggers type I IFN production by dendritic cells (192–195) and the cascade towards chronic immune activation observed in HIV patients (196, 197). Acute type I IFN exposure has been shown to induce proliferation of c-Kit^+ HSPCs in mice, whereas chronic type I IFN treatment led to HSPC apoptosis due to proapoptotic induction by IFN exposure irrespective of duration (198). The presence of HIV-infected dendritic cells in the bone marrow

therefore contributes to haematopoietic dysfunction by directly affecting HSPCs (199).

While monocyte-derived tissue-resident macrophages do not return to the bone marrow, macrophages support viral replication and are important members of the viral reservoir (200) in combination with circulating CD4+ T-cells (201). Circulating CD4 + T-cells are present at higher rates in the bone marrow of HIV positive individuals compared to HIV negative individuals (202), exacerbating the effects of aberrant T-cell cytokine production on the bone marrow. During HIV infection, increased production of IL-4 by T-helper 2 (Th2) CD4+ T-cells was observed (203), which was reported to impair megakaryocyte production in leukaemia (204) and could reasonably be expected to contribute to thrombocytopenia.

It has been suggested that a population of resident memory T-cells in the bone marrow niche may play a role in long-lived immunity against systemic pathogens (205–208), although this is not yet fully understood (209, 210). Resting CD4+ memory and T-cells are well-described as an important latent reservoir for HIV, extensively reviewed elsewhere (206, 211). Bone marrow CD4+ memory T-cells were found to harbour similar levels of virus to circulating CD4+ T-cells in simian immunodeficiency virus (SIV)-infected rhesus macaques (212) and HIV-infected individuals (211). The susceptibility of these cells to HIV infection and their resident status in the bone marrow presents a source of HIV and HIV proteins in the bone marrow outside of circulating infected cells. Activation of this latent reservoir in the bone marrow could result in an increase in HIV load in the bone marrow, which may infect surrounding cells and cause dysregulation of bone marrow niche cells as a consequence of the presence of HIV proteins.

Interactions between cells in the bone marrow are critical for normal haematopoiesis as well as HSPC maintenance and regulation. Bone marrow homeostasis is disrupted during HIV infection as a consequence of direct infection of niche cells and/or the effects of HIV proteins. As a consequence of HIV infection or the effects of HIV proteins on perivascular niche cells, perivascular niche HSPCs are driven towards quiescence and mobilisation, thereby impairing haematopoiesis. Endosteal niche HSPCs are driven to mobilisation due to the breakdown of normal bone remodelling and MSC senescence resulting from HIV infection. The consequences of the indirect effects of HIV infection on HSPCs are therefore deleterious, ultimately reducing the number of HSPCs in the bone marrow and the creation of a quiescence-supporting environment for the remaining HSPCs.

Direct effects of HIV on HSPCs

The indirect effects of HIV infection on HSPCs are complex and cumulative, contributing to the impairment of HSPC function. The direct effects of HIV on HSPCs encompass both direct infection and the effect of HIV proteins on HSPC function. However, the literature presents opposing conclusions regarding the susceptibility of HSPCs to HIV infection.

HSPCs in the bone marrow are directed to remain quiescent or divide and differentiate, forming blood and immune cells in response to the cytokine milieu. At key points, differentiating HSPCs will become lineage restricted and only form the cell types dictated by the cytokine milieu. This means that one infected HSPC would produce a limited number of haematopoietic cell types harbouring HIV. Nixon et al., 2013 demonstrated that HSPC progeny generated through colony-forming assays of CD34+ cells from HIV-infected humanised mice, harboured HIV. Clonally infected cells resulting from HSPC division and differentiation would therefore be restricted to a single or limited number of haematopoietic cell types, depending on the differentiation potential of the infected HSPC and on external stimulus directing haematopoiesis. Transcriptional activation during differentiation could activate HIV replication from integrated or episomal provirus, possibly resulting in cell death due to the lytic nature of HIV replication. Carter et al., 2010 (213) showed that CD34+ cells expressing HIV gene products were markedly depleted in culture compared to a transduced control, which could indicate some other mechanism of cell death in infected HSPCs. *In vivo*, host cell lysis or death might contribute to the absence of terminally differentiated HIV-infected cells of all haematopoietic lineages harbouring clonal virus initially of HSPC-origin. HIV infection may skew haematopoiesis towards or away from certain lineages (214, 215), which may contribute to cytopenia and the lack of clonal infection of certain haematopoietic cell types. This is not well described in literature as bone marrow research is limited to static snapshots of a highly dynamic environment.

The controversy in literature dates back to the early 1990s with Stanley and colleagues detecting HIV in CD34+ HSPCs from seropositive patients (216) and Neal and colleagues presenting alternate data showing that CD34+ HSPCs were rarely infected with HIV in asymptomatic patients (217). This was followed by a number of studies with different conclusions and one paper suggesting that HIV-1 subtypes may differ in their ability to infect HSPCs (218). Several studies found HSPC subsets to be resistant to HIV infection (217–230), the suggested mechanism being through a p21-mediated pre-integration block (231). Given the inducible expression of HIV proteins in the presence of a pre-integration block, the findings may suggest transient transcription from episomal proviral DNA in HSPCs (213). In contrast, a number of studies have detected HIV in HSPCs (213, 214, 216, 218, 222, 226, 229, 230, 232–236). Carter and colleagues showed latent infection of Lin-CD34+CD133+CD38- primitive HSPC subsets *in vitro* and corroborated these findings with bone marrow CD34+ HSPCs from HIV infected individuals with high viral load (213). However, their findings also suggest that HIV-infected cells actively expressing HIV proteins were short-lived compared to their latently-infected counterparts (213). Several follow-up studies reported similar findings (214, 230, 235, 236), which are presented in Table 1. A recent study reported that a small subset of the heterogenous CD34+ HSPC population expresses low levels of CD4, and that this subset was found to harbour HIV genomes *in vivo* (237). While detection of HIV in this subset is not necessarily surprising, the fact that both R5- and X4-tropic HIV genomes were detected was notable as CXCR4 is usually expressed in a greater fraction of CD34+ HSPCs than is CCR5 (213). While HSPC susceptibility to HIV infection hinges largely on the expression of CD4, CXCR4, and CCR5, CD4-independent infection mechanisms

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TABLE 1 Comparison of literature on the susceptibility of HSPCs to HIV infection.

HIV DETECTED							
Cells			Infection		HIV detection		Reference
Source	Phenotype	Activation method	HIV	*In vitro/ vivo*	Method	Target	
BM	CD34+	NA	Uncharacterised HIV+ donors (Zaire and North America)	*In vivo*	PCR	*env*, *gag*	Stanley et al., 1992 (216)
PB	CD34+/- (BFU-E and CFU-GM colonies)	Overnight pre-stimulation in SCF, IL-3, GM-CSF, Epo	X4-tropic HIV-1B molecular clone	*In vitro*	RT-PCR, ELISA	*tat*, *gag* p24	† Chelucci et al., 1995 (226)
BM	CD34+, CD34+CD38+, CD34+CD38-,	NA	R5- and X4-tropic HIV-1 molecular clones. Uncharacterised patient virus (USA)	*In vitro; in vivo*	PCR, ELISA	*gag*, *LTR* p24	Shen et al., 1999 (229)
PB, UCB	CD34+, MNCs	Pre-cultured with SCF, GM-CSF, IL-3, Epo	R5-tropic HIV-1C molecular clones and primary HIV+ patients (Botswana)	*In vitro, in vivo*	RT-PCR, ELISA	*gag* p24	† Redd et al., 2007 (218)
BM, UCB	CD34+, CD133+	NA	Uncharacterised patient virus; R5X4-tropic HIV-1B molecular clones and pseudovirus	*In vitro, in vivo*	Flow cytometry, qPCR	Gag (KC57 and anti-p24 mAB), *LTR*	‡ † Carter et al., 2010 (213)
BM, UCB	CD34+, CD133+	Pre-stimulation in SCF, TPO, FLT3-L, IGFBP-2	X4- and R5X4-tropic HIV-1B molecular clones and pseudotyped viruses	*In vitro, in vivo*	Flow cytometry	GFP, IC Gag	‡ Carter et al., 2011 (230)
BM	CD133+, CD34+CD45RA-CD38-	Pre-stimulation in SCF, TPO, FLT3-L, IGFBP-2	Pseudotyped virus	*In vitro*	Flow cytometry	GFP, PLAP, p24	‡ McNamara et al., 2012 (235)
BM	CD133+	NA	Uncharacterised HIV+ donors on ART with plasma viral loads of <48 copies/ mL.	*In vivo*	qPCR	*Gag*, *LTR*	* McNamara et al., 2013 (236)
UCB, fetal liver	CD34+CD38+CD123+ (CMP), CD34+CD38 +CD45RA+ (GMP) and CD34+CD38+CD110+ (MEP)	NA	Wild-type viruses were created from proviral plasmids p89.6, pYJRCSF, and pNL4-3.	*In vitro*	qRT-PCR	*Gag*, *LTR*	‡ Nixon et al., 2013 (214)
PB, BM	Lin-CD34+	NA	Uncharacterised HIV+ donors (naïve and on ART)	*In vivo*	qPCR	*LTR*	* Bordoni et al., 2015 (232)
BM	Lin-CD34+	NA	HIV-infected humanized mice (5 – 14 weeks post-infection)	*In vivo*	qPCR, Immunofluorescence	*Gag*	Araínga et al., 2016 (233)
BM	CD34+, CD133+	UCB-derived cells pre-cultured for 4 days	X4- and R5-tropic HIV-1B molecular clones. Uncharacterised HIV+ donors	*In vitro, in vivo*	Flow cytometry, PCR	*Gag*, *env*	* Sebastian et al., 2017 (237)
BM	CD133+, CD34+CD133-	NA	Uncharacterised HIV+ donors	*In vivo*	PCR	*Gag*, *env*	* Zaikos et al., 2018 (234)
BM, CB	Lin-, CD34+CD38-CD45RA-Lin-, Lin-CD34 +CD38-CD45RA-CD90-, CD34+CD38-CD45RA-CD90+, CD34+CD38+	NA	X4-tropic pseudotyped GFP reporter viruses, X4- and R5-tropic HIV-1B molecular clones. Uncharacterised HIV + donors, one donor with confirmed HIV-1B infection	*In vitro; in vivo*	Flow cytometry, qPCR	GFP, p24, *HIV-1 R-U5/ gag*	* Renelt et al., 2022 (222)

(Continued)

TABLE 1 Continued

HIV NOT DETECTED							
Cells			Infection		HIV detection		Reference
Source	Phenotype	Activation method	Virus	*In vitro/ vivo*	Method	Target	
BM	Colony-forming cells from T-cell and adherent BM cell-depleted BM fractions	NA	Uncharacterised HIV+ donors (North America); HIV-1B and HIV-2A (isolate ROD)	*In vivo; in vitro*	PCR	*Gag*	Molina et al., 1990 (223)
BM	CD34+	NA	Uncharacterised HIV+ donors (North America)	*In vivo*	PCR	*Env*, *gag*	Davis et al., 1991 (224)
BM	CD34+	NA	Uncharacterised HIV+ donors (France)	*In vivo*	PCR, Flow cytometry	*gag* p24, gp120	Louache et al., 1992 (225)
PB	CD34+/- (CFU-GEMM)	Overnight pre-stimulation in SCF, IL-3, GM-CSF, Epo	X4-tropic HIV-1B molecular clone	*In vitro*	RT-PCR, ELISA	*Tat*, *gag* p24	Chelucci et al., 1995 (226)
BM	CD34+, CD34-, MNCs	NA	Uncharacterised HIV-1+ donors (USA) on ART with no AIDS-defining illness	*In vivo*	PCR	*Gag*, *pol*	Neal et al., 1995 (217)
BM	CD34+CD38-; CD34+CD4+	NA	Uncharacterised HIV+ donors (France)	*In vivo*	PCR	*gag*	Marandin et al., 1996 (227)
BM	CD34+CD38-; CD34+CD38+	NA	R5 and R5X4-tropic HIV-1 and R5X4 HIV-2 molecular clones	*In vitro*	PCR, ELISA	*gag* p24	Weichold et al., 1998 (228)
BM	G0 CD34+	7-day pre-culture	R5- and X4-tropic HIV-1 molecular clones. Uncharacterised patient virus (USA)	*In vitro; in vivo*	PCR, ELISA	*gag*, *LTR* p24	Shen et al., 1999 (229)
PB, UCB	CD34+, MNCs	Pre-cultured with SCF, GM-CSF, IL-3, Epo	R5-tropic HIV- 1B molecular clones and primary HIV (USA)	*In vitro; in vivo*	RT-PCR, p24 ELISA	*gag*	Redd et al., 2007 (218)
BM, UCB	CD34+, CD133+	Pre-stimulation in SCF, TPO, FLT3-L, IGFBP-2	R5- tropic HIV-1B molecular clones; pseudotyped viruses; HIV+ donors	*In vitro, in vivo*	Flow cytometry	GFP, IC *Gag*	Carter et al., 2011 (230)
BM	CD34+	Pre-cultured with SCF, TPO, FLT3-L and GM-CSF and TNF-α, or PMA	HIV+ donors (Patients on ART and VL <50 copies/mL)	*In vivo*	PCR	*gag*	Durand et al., 2012 (219)
BM	Lin-CD34+, Lin-CD34-	NA	HIV-1B+ donors (Patients on ART and VL <45-70 copies/mL)	*In vivo*	PCR	Target not specified	Josefsson et al., 2012 (220)
UCB	CD34+	24 hr pre-stimulation with TPO, SCF, and FLT3-L	VSV-G-pseudotyped virus with a modified pNL4.3 HIV-1-based core including an mCherry ORF	*In vitro*	Flow cytometry, qPCR	mCherry	Griffin & Goff, 2015 (221)
BM	Lin-CD34+CD38-CD45RA-CD90-, CD34+CD38-CD45RA-CD90+	NA	R5-tropic pseudotyped GFP reporter viruses	*In vitro*	Flow cytometry	GFP	Renelt et al., 2022 (222)

BM, bone marrow; PB, peripheral blood; UCB, umbilical cord blood; BFU-E, burst-forming unit erythroid; CFU-GM, colony-forming unit granulocyte-macrophage; CMP, common myeloid progenitor; GMP, granulocyte-macrophage progenitor; MEP, megakaryocyte-erythroid progenitor; CFU-GEMM, colony-forming unit granulocyte-erythroid-macrophage-megakaryocyte; SCF, stem cell factor; IL-3, interleukin-3; GM-CSF, granulocyte-macrophage colony-stimulating factor; Epo, erythropoietin; IGFBP-2, insulin-like growth factor binding protein-2; TNF-α, tumour necrosis factor alpha; PMA, phorbol myristate acetate; FLT3-L, fms-like tyrosine kinase receptor 3 ligand; TPO, thrombopoietin; R5, C-C-motif chemokine receptor type 5; X4, C-X-C,motif chemokine receptor type 4; PCR, polymerase chain reaction; qPCR, quantitative PCR; RT-qPCR, real-time qPCR; ELISA, enzyme-linked immunosorbent assay; IC, intracellular; GFP, green fluorescent protein.
*T-cell contamination was robustly excluded from analysis (<1% T-cells).
‡T-cell contamination unlikely due to single-cell HIV-detection by flow cytometry.
†T-cell contamination unlikely due to culture conditions (CFU assays).

have been described (238–241) and should not be discounted for infection of HSPCs. Most recently, Renelt and colleagues made a strong argument for HIV infection of CD133+ and CD34+CD133- HSPC subpopulations in some donors, and their contribution to viremia using proviral sequence tracing (222).

A closer inspection of studies exploring the susceptibility of HSPCs to HIV infection outlined in Table 1 reveals that different experimental approaches may in part explain the lack of consensus between studies to some degree. Variations in culturing, the use of growth factors, HIV moieties, infection strategy, and HIV detection method could contribute to variation between results. These factors and how each could affect the outcome of the study are discussed in more detail below.

HSPCs are rare cells that often require *in vitro* expansion so that enough cells are obtained to optimally perform experiments. Numerous studies have cultured or expanded HSPCs for several days before *in vitro* infection with HIV. Expression of CXCR4 is upregulated on murine HSPCs after overnight incubation (242), which may artefactually increase susceptibility to CXCR4-tropic HIV. Similarly, HIV integration and replication is dependent on the activation state of target cells (243) with dividing cells being more susceptible to productive HIV infection. The majority of HSPCs (>90%) are in a quiescent state *in vivo* (244) in the bone marrow niche; expansion prior to infection would therefore create an *ex vivo* artefactual state. Expansion and culturing of HSPCs *in vitro* is often performed in the presence of haematopoietic cytokines which promote expansion and HSPC survival in culture. However, there is not currently a standardised cytokine cocktail for HSPC expansion and maintenance. Studies investigating the susceptibility of HSPCs to HIV infection have been performed with (218, 226, 230, 235) and without (229, 231, 245) cytokines. The duration of culture and the supplementation of medium with cytokines could therefore result in increased susceptibility of HSPCs to HIV infection that is not inherent but rather an artefact of culturing.

The use of HIV propagated *in vitro* in the form of laboratory-generated HIV molecular clones, pseudo- or pseudo-typed virus, or cultured primary virus has several aspects where outcome-critical variation between studies could occur. The most glaring differences between studies are (i) multiplicity of infection (MOI), (ii) infection strategy, and (iii) nature of virus used. Unrealistic bombardment of target cells with extremely high MOIs, referring to the number of infectious units per target cell, could result in artefactual infection *in vitro*, which is unlikely *in vivo*. The infection strategy is similarly crucial to a translatable experimental outcome. Infection in small volumes or using centrifugal force (termed "spinoculation") to create close contact between cells are two methods commonly used to increase the potential for infection. Spinoculation is not a physiological condition and could therefore also result in artefactual infection. The nature of the virus used in experiments is constrained by several factors including but not limited to biosafety, availability of comparable research tools, and the effect of HIV proteins on target cells which can affect results. As previously mentioned, HIV-1B (being the most-studied subtype) epitomises what is known about HIV infection. However, distinct characteristics including coreceptor usage during early and late infection (246) and reduced cytopathic effects (58) have been documented for HIV-1C, which could affect research outcomes in terms of latency and host cell susceptibility. Variation from HIV-1B has been reported for non-B subtypes in several aspects related to viral fitness and disease progression (45–48, 50, 51, 55, 58, 59, 61, 218, 247–250) which are outside the scope of this review but are important when comparing research findings.

The method used to detect HIV is another critical factor to be considered when comparing studies. In addition to the increased sensitivity that comes with improvements in detection technologies over time, studies have varied with the technology used to detect the presence of HIV in target cells. Proviral DNA, viral transcripts, or viral proteins can be targeted, and each detection method comes with a limit of detection and considerations for use. This is particularly important where, as with HSPCs, it is reasonable to expect low to very low proportions of infected cells. This is illustrated in a study by Izopet et al. (251) who were able to detect four proviral genomes per million cells and found that the frequency of infection of highly susceptible CD4+ T-cells *in vivo* can be lower than 1%, as reported in other studies (252–254). The sensitivity of the HIV detection method is often not reported, and the improvements of technologies over time are difficult to categorise, but these are equally important to consider when comparing older and more recent studies.

Disruption of HSPC function can also be caused by the presence of HIV proteins. The HIV receptor protein gp120 has been shown to impair the clonogenic potential of HSPCs and induce apoptosis through Fas-dependent endogenous TGF-β upregulation (255). Suppression of HSPC colony formation is caused by HIV-1 p24 (256). Exposure to HIV Tat protein stimulates TGF-β production in macrophages resulting in myelosuppression *in vitro* (255), and viral protein R (Vpr) has been shown to induce phagocytosis of bone marrow cells by mononuclear phagocytes (257). Blocking TGF-β in purified CD34+ HSPCs exposed to HIV reportedly improved growth and survival (255), and this is supported by the simultaneous downregulation of a proliferation-inducing ligand (APRIL) with TGF-β upregulation induced by exposure to gp120 (258). Furthermore, Nef has been shown to act as a PPARγ agonist with deleterious effects on early haematopoiesis in macaques (259). Cumulatively, the effects of HIV proteins in the bone marrow are deleterious to HSPC and niche cell function and survival, and ultimately contribute to haematological abnormalities present in HIV patients independently of direct HSPC infection.

The conditions under which HSPCs may become susceptible to HIV infection in the bone marrow are not clear based on current information from the literature; what is clear however is that HIV proteins have a direct suppressive effect on HSPC function.

Concluding remarks

Haematopoietic dysfunction in HIV patients is well-documented and results from the combined direct and indirect effects of HIV on HSPCs. The bone marrow niche is a uniquely complex environment which is yet to be fully understood. Healthy human bone marrow is therefore not completely represented in literature, which makes it difficult to fully model the marrow under conditions of HIV infection. The limited understanding of *in vivo*

susceptibility of bone marrow cells to HIV and the fact that bone marrow cell types have largely been studied *in vitro* or in animal models contribute to the paucity of literature on the HIV-infected marrow. Moreover, the evolution of HIV detection methods over time, and the understanding that detection of HIV proteins or partial DNA does not necessarily indicate productive infection, compound this challenge. Although it is undeniable that HIV affects haematopoiesis, the susceptibility of HSPCs to HIV has long been debated. Studies investigating HIV infection in HSPCs differ critically in methodology and HSPC subpopulations used. This review has aimed to highlight what is currently known about the consequences of HIV infection on the bone marrow niche, and to summarise the studies to date which have attempted to determine the susceptibility of HSPCs to HIV infection. This is particularly relevant to the fields of stem cell transplantation and HIV pathogenesis, and potentially to the treatment of HIV-associated haematological malignancies.

Author contributions

CH and JM wrote, compiled, and edited the manuscript. CH made the figures. TM contributed to the manuscript. MP and CD were involved in manuscript conception. MP edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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