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Neutropenia during HIV Infection: Adverse Consequences and Remedies

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Abstract

Neutropenia frequently occurs in patients with Human immunodeficiency virus (HIV) infection. Causes for neutropenia during HIV infection are multifactoral, including the viral toxicity to hematopoietic tissue, the use of myelotoxic agents for treatment, complication with secondary infections and malignancies, as well as the patient's association with confounding factors which impair myelopoiesis. An increased prevalence and severity of neutropenia is commonly seen in advanced stages of HIV disease. Decline of neutrophil phagocytic defense in combination with the failure of adaptive immunity renders the host highly susceptible to developing fatal secondary infections. Neutropenia and myelosuppression also restrict the use of many antimicrobial agents for treatment of infections caused by HIV and opportunistic pathogens. In recent years, HIV infection has increasingly become a chronic disease because of progress in antiretroviral therapy (ART). Prevention and treatment of severe neutropenia becomes critical for improving the survival of HIV-infected patients.

Keywords

AIDS; granulocytopenia; HAART; myelosuppression; neutrophil

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Declaration of Interest

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INTRODUCTION

Human immunodeficiency virus (HIV) infection causes abnormality of CD4+ T cell function and loss of these immune effector cells through direct and indirect cytotoxicities of the virus. HIV-induced destruction of the adaptive immune system leads to progressive deterioration of host immune defense and the development of acquired immunodeficiency syndrome (AIDS) in infected individuals [1]. In association with the decline of adaptive immunity, myelosuppression, characterized by neutropenia (or granulocytopenia), anemia and thrombocytopenia frequently occurs in patients with HIV infection [2–4]. In particular, the occurrence of severe neutropenia superimposed upon the collapse of adaptive immunity greatly increases the risk of fatal secondary infections [5–8]. Since neutropenia is a dose-limiting factor for several antimicrobial agents, such neutropenia may also interfere with the treatment of primary HIV infection and associated secondary infections. This article discusses the recent developments in understanding the etiology and pathogenesis of neutropenia during HIV infection. Current progress in the prevention and treatment of this health problem is also evaluated.

Historic Overview of Epidemiology

Abnormality of neutrophil (granulocyte, polymorphonuclear leukocyte or PMN) counts in the peripheral circulation was observed in patients with HIV infection at the beginning of recognizing this viral disease. As early as in 1983, when HIV was identified as the pathogen of AIDS [9, 10], leucopenia and decreased lymphocyte counts in the peripheral blood were reported in three of seven male prisoners with AIDS from New York State correctional facilities [11]. In that same year, a case report of an AIDS patient described pancytopenia along with hypocellular features in the bone marrow [12]. In 1984, a clinical study of hematological abnormalities in 12 patients with AIDS reported that eight patients exhibited leucopenia [13]. Besides lymphopenia, the most common peripheral blood abnormalities were a left shift in the granulocyte series and vacuolated monocytes. Bone marrow examination showed a left shift in the myeloid series in most of these patients. In 1985, a study of 93 homosexual men with persistent lymphadenopathy treated at the Memorial Sloan-Kettering Cancer Center found that over 90% of those individuals were positive for serum anti-HIV antibodies. In addition to lymphopenia, granulocytopenia and monocytopenia were common clinical findings [14]. At the same time, another report of eight patients with AIDS described granulocytopenia in six of them during hospitalization. The other two had low-normal neutrophil counts. Bone marrow examination exhibited abnormalities in maturation of all cell lineages with the most prominent abnormality in the granulocytic series, similar to findings in the myelodysplastic syndrome (preleukemia) [2].

In 1987, an early comprehensive analysis of hematology in 102 cases of AIDS, AIDS-related complex (ARC, a historical term) and asymptomatic homosexual men at risk for and/or infected with HIV reported neutropenia in 30% of healthy homosexual males with serum antibodies to HIV [15]. This study also revealed that the prevalence of anemia, granulocytopenia and thrombocytopenia paralleled the severity of the HIV disease. Bone marrow examination demonstrated frequent dysplasia, plasmacytosis and lymphoid infiltrates. These marrow morphologic findings were strongly associated with anemia and

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granulocytopenia. Two other studies of HIV disease in the same year also described low-circulating neutrophil counts in 55% to 83% of patients [16, 17]. Bone marrow biopsies revealed a myelodysplastic appearance in most of these individuals. Shortly thereafter, more clinical studies observed the frequent association of neutropenia with the progression of HIV disease. In 1989, an investigation of peripheral blood and bone marrow changes in 60 patients with HIV disease found that hematological abnormalities were most commonly seen in patients with advanced HIV disease. The highest incidence of neutropenia (85%) was observed in AIDS group IV patients (CDC classification) [18]. Concomitantly, a report of bone marrow examinations in 20 patients with AIDS and 39 patients with AIDS-related complex showed that leucopenia most frequently occurred in patients with AIDS [19]. In that series of bone marrow samples, HIV nucleic acids were detected in 11 cases. Precursors along myeloid lineages including immature myeloid cells and nucleated red blood cells were positive for HIV nucleic acids in some instances [19].

Since then, the epidemic characteristics of neutropenia during HIV infection have been more clearly identified through large cohort patient studies in various medical settings. In 1999, a 10-year (1982–1993) database analysis of 1870 patients with HIV infection in Brussels, Belgium reported that 484 patients had developed neutropenia [8]. In 2006, a study of 533 sub-Saharan African adult patients with HIV infection in Abidjan documented that blood neutrophil counts lower than 1500 cells/μL occurred in 66% of patients during a median follow-up period of 29 months [20]. A recent multicenter prospective study examining the manifestation and course of HIV infection in US females reported that in 1729 HIV-infected women, 79% had blood neutrophil counts less than 2000 cells/μL and 31% experienced neutrophil counts below 1000 cells/μL during a 7.5 year follow-up period [21].

Clinical Manifestations

Generally, the neutrophil count in the peripheral blood of a normal adult is greater than 2.0×10^9 cells/L (or 2,000 cells/μL) [16, 21, 22]. The definition of neutropenia during HIV infection varies among reports from different health care settings. Toure and colleagues have defined grades of neutropenia in their HIV-infected patients as at least one absolute neutrophil count (ANC) of: <1500 cells/μL (severity grade ≥1), <1000 cells/μL (grade ≥ 2), <750 cells/μL (grade ≥ 3) or <500 cells/μL (grade 4) [20]. Clinical manifestations of neutropenia in patients with HIV infection are complex. The occurrence of neutropenia during the course of HIV infection differs in duration and severity. Circulating neutrophil counts usually diminish progressively as the HIV disease advances [15, 18, 23]. Neutropenia frequently develops along with pancytopenia in patients infected with HIV. In particular, a decreased blood neutrophil count is closely associated with the loss of CD4+ T cells and is inversely correlated with the HIV viral load in the circulation [24, 25]. A study of 244 HIV-infected patients with neutropenia (absolute neutrophil count < 1000 cells/μL) has shown that neutropenia occurs at a median CD4+ cell count of 30 cells/μL. Low CD4+ cell count is associated with longer episodes of neutropenia and with a more profound nadir. Two-thirds of the neutropenic episodes last less than 2 weeks. Absolute neutrophil count nadir is < 500 cells/μL in 45% of episodes [26].

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Although neutropenia is commonly seen in the late stage of HIV disease, exceptions do exist. In a small number of patients, a decreased neutrophil count can occur during the initial period of HIV infection [27–30]. In some specific populations, such as pediatric patients, peripheral cytopenias including neutropenia may even appear as the first sign of HIV infection [29]. As mentioned above, left shift in the granulocytic series is frequently seen in HIV-infected patients with neutropenia. Bone marrow features of these patients include myelodysplasia with a left shift in the myelold series, abnormalities in maturation of cells along the granulocyte lineage, lymphocytic infiltration, and plasmocytosis [2, 13, 30–33]. Resolution of neutropenia can be achieved in association with the restoration of CD4+ cell count and the decline in HIV viral load in the circulation following treatment with highly active antiretroviral therapy (HAART) [21].

In addition to neutropenia, abnormalities of neutrophil function have been observed in HIV-infected patients. These abnormalities include reduced bactericidal capacity, defective degranulation process, impaired chemotaxis, ineffective phagocytosis, abnormal surface adhesion molecule expression and reduced production of toxic oxygen species [25, 34–37]. Neutrophils are critically important in host defense against bacterial and fungal pathogens [6]. Decreases in the number of neutrophils and functional abnormalities of the remaining neutrophils in patients with HIV infection severely impairs the host ability to kill bacterial and fungal invaders, particularly when the adaptive immunity has also been compromised by HIV toxicity to CD4+ T lymphocytes.

Clinical studies have shown that neutropenia and defects in innate immunity are risk factors for developing secondary infections caused by bacterial and fungal pathogens in HIV-infected individuals [6, 38]. Serious secondary infections most often occur at the nadir of the absolute neutrophil counts [26, 39]. An evaluation of 2047 patients from the outpatient HIV clinic at San Francisco General Hospital has shown that a significantly higher risk of hospitalization for bacterial infections is associated with absolute neutrophil counts lower than 0.75×10^9 cells/L (750 cells/μL), especially those lower than 0.5×10^9 cells/L (500 cells/μL) [5]. Another matched case-control study of 1870 patients with HIV infection has reported that the incidence of severe bacterial and fungal infections is significantly higher in neutropenic patients [8]. HIV-infected patients with neutropenia are at significant risk of developing severe secondary infections in the end-stage of HIV disease, which exerts a major impact on hospitalization and death. Indeed, neutropenia has been identified as an independent risk factor for both the development of bacteremia in individuals infected with HIV [40–42] and the increase in mortality of HIV-infected patients suffering from bloodstream infections [24, 43]. Conversely, treatment of neutropenia in patients with HIV infection improves their survival [44].

One mechanism by which neutrophils contribute to pathogen clearance is the production of neutrophil extracellular traps (NETs), genomic DNA-based net-like structures capable of capturing invading pathogens. NETs capture HIV and promote HIV elimination through mechanisms involving myeloperoxidase and α-defensin [45]. Neutrophils detect HIV by toll-like receptors (TLRs) 7 and 8, which recognize viral nucleic acids. Engagement of TLR7 and TLR8 induces generation of reactive oxygen species that trigger NET formation, leading to NET-dependent HIV elimination. It remains to be determined whether

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myelosuppresion and neutrophenia impair host anti-HIV defense during the course of HIV infection.

Several therapeutic agents for HIV and opportunistic infections, including zidovudine (AZT), cotrimoxazole, cidofovir, foscarnet, ganciclovir and trimethoprim/sulfamethoxazole are myelotoxic. Myelosuppression is a dose-limiting side effect of these antimicrobial compounds. Development of severe neutropenia in patients may require (at least temporarily) stopping use of these agents during HIV treatment [46]. Therefore, neutropenia may negatively affect HIV disease progression by interfering with antiretroviral therapy.

In some HIV-infected individuals, however, neutropenia can be transient without a clear correlation with the development of secondary infections [20]. Reasons for these inconsistent observations remain to be determined. Multiple factors, including stages of disease among tested subjects, sample sizes of patients studied, geographic locations of patient cohorts, quality control criteria for data collection and analysis, as well as the identity of therapeutic agents used for the treatment of the primary viral infection, likely influence conclusions drawn from various studies.

Etiology and Pathogenesis

There are many causes for neutropenia during HIV infection, including HIV infection itself, HIV-related autoimmune disorders, secondary infections, malignancies, agents used to treat HIV and opportunistic infections, and confounding factors associated with patients suffering from HIV infection. Malnutrition may also contribute to the development of neutropenia in individuals infected with HIV. Table 1 summarizes diverse causes of neutropenia in HIV infection.

HIV toxicity to hematopoietic tissue—Development of neutropenia has a close correlation with the viral load in HIV-infected patients [21, 24, 25], which suggests that HIV may have cytotoxicity to cells of the granulocyte lineage and/or their ancestors. At the present time, there is no evidence that HIV destroys mature neutrophils through direct viral infection. As neutrophils have a relatively short lifespan in the systemic circulation, maintenance of normal neutrophil levels in the peripheral blood essentially relies on daily production of sufficient neutrophils from the hematopoietic tissue. In the clinic, neutropenia frequently occurs along with pancytopenia that involves all myeloid lineages in HIV-infected patients. Abnormality of common ancestor cells, such as hematopoietic stem cells (HSCs) or early myeloid progenitors, most likely plays an important role in the pathogenesis. The function of hematopoietic stem/progenitor cells (HSPCs) can be compromised during HIV infection either through the direct toxicity of HIV to primitive hematopoietic precursor cells or via the indirect effect of the altered marrow niche environment [19, 47–50]. In humans, the CD34+ hematopoietic cell population contains many types of HSPCs ranging from HSCs with extensive self-renewal capacity to progenitor cells committed to lineage differentiation [51]. Certain CD34+ cells express HIV receptors (CD4, CXCR4 and CCR5) making them potentially susceptible to HIV-1 infection [52–56]. HIV nucleic acids have been detected in precursors along myeloid lineages in patients with AIDS [19]. *Gag*-coded HIV-1 proteins have also been identified in CD34+ progenitor cells from HIV-1-infected

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patients [57]. In addition, several isolates of HIV-1C can infect CD34+ cells *in vitro* [58]. Indead, HIV proviruses can be detected in CD34+ cells from the peripheral blood of individuals infected with HIV-1C. The level of HIV detected in CD34+ cell samples is greater than that observed in total peripheral blood mononuclear cells from the same patients, eliminating the potential for mononuclear cell contamination in CD34+ HSPC fractions. Flow cytometric analysis of HIV protein expression in CD34+ cells following exposure to HIV has shown that a variety of HIV strains, including several HIV-1B isolates, can infect CD34+ cells derived from human bone marrow or umbilical cord blood [59]. Both active and latent infections of CD34+ cells have been detected in HIV positive individuals. HIV-1 genomes have also been found in CD34+ cells from patients with well-controlled viremia on HAART. In light of these discoveries, marrow HSPCs are now considered as a cellular reservoir of HIV infection [47].

Mechanisms underlying HIV cytotoxicity to HSPC remain incompletely understood. Multiple factors appear to be involved in mediating HIV cytotoxicity to HSPCs and the resultant myelosuppression. Both the viral load and the biological characteristics of the virus appear to play an important role in inducing the suppression [60]. *In vitro* studies have demonstrated that HIV is cytotoxic to infected HSPCs, leading to death of these hematopoietic precursors [59]. Death of infected CD34+ cells appears to require active viral gene expression. Transduction of HSPCs with a reporter virus pseudotyped with an HIV envelope does not cause cell loss unless the HIV LTR actively expresses HIV genes [59]. Other reports have indicated that heat-inactivated HIV-1 and cross-linked envelope glycoprotein gp120 induce a decrease in clonogenic capacity, impairment of cell cycling and apoptosis in CD34+ HSPCs through a Fas-dependent mechanism [61, 62]. HIV and HIV protein gp120 can also suppress CD34+ cell growth through induction of the endogenous growth inhibitory cytokine TGF-β [61]. Clonogenic assays have shown that proliferation of granulomonocytic progenitor cells (CFU-GM) is inhibited by HIV negative factor (Nef) [63]. Conditioned medium from HIV-1 nonproductively infected liquid cultures inhibits the proliferation of CFU-GM cells. This inhibitory effect can be neutralized by specific anti-Nef antibodies. Recombinant Nef possesses the same growth inhibitory property. Soluble Nef can activate the transcriptional suppressor PPARγ in uninfected CD34+ cells. PPARγ suppresses the expression of STAT5A and STAT5B, two factors necessary for proper function of primitive hematopoietic precursors [64]. HIV Gag p24 has been reported to inhibit CFU-GM activity in CD34+ cells through a receptor-mediated mechanism [65]. Tat has also been reported to impair myeloid development in the bone marrow, suggesting that a complex array of HIV proteins mediate myelosuppression during HIV infection [66]. Consistent with these studies, bone marrow examinations of HIV-infected patients have confirmed that there is a marked reduction in HSPC self-renewal or proliferation as reflected by a significant decrease in expression of the cell cycling-associated nuclear antigen recognized by the Ki67 antibody [57]. Decreases in the number of primitive hematopoietic precursor cells have been observed in patients infected with HIV and in nonhuman primates infected with simian immunodeficiency viruses (SIV) [67–70]. Bone marrow and/or blood CD34+ cells from HIV-infected patients exhibit reduced capacity for growth and differentiation [71, 72]. Significantly fewer CFU-GM exist in the peripheral blood of patients with AIDS [73]. The number of circulating CFU-GM is inversely correlated with

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the presence of Gag p24 in the plasma and with the viral recovery from blood mononuclear cells. HIV-infected individuals have a marked decrease in CD34+/CD38− and CD34+ Thy-1+ cell fractions, which suggests that phenotypically primitive hematopoietic precursor cells are depleted during HIV infection [71, 74]. In SIV-infected rhesus macaques, the number of CD34+ cells and CFU-GM progenitor cells in the bone marrow is decreased in the advanced stage of the disease [75]. Consistent with these observations, filgrastim (recombinant human granulocyte colony-stimulating factor or rhG-CSF) induced mobilization of marrow CD34+ cells into the peripheral blood is significantly reduced in patients with advanced HIV disease [76].

HIV can infect different cell types in the marrow hematopoietic niche environment. HIV-derived molecules are also able to stimulate these niche cells to alter their production of cytokines and growth factors. The bone marrow stroma provides a complex network for cell–cell interactions and a soluble mediator milieu to support survival, proliferation, and differentiation of HSPCs. Bone marrow hematopoietic niche cell types include fibroblasts, endothelial cells, osteoblasts, reticular stromal cells, adipocytes and certain mature hematopoietic cell types such as macrophages, lymphocytes and megakaryocytes [77–80]. HIV infection causes changes in the bone marrow stromal structure characterized by a decrease in the fibroblastic population and an increase in macrophage-like cells [81]. In some cases, these macrophage-like cells are positive for HIV DNA, suggesting that they are a target of HIV infection. *In vitro* studies have shown that primary human bone marrow stromal fibroblasts are susceptible to HIV infection [82]. Infected marrow fibroblasts can serve as the HIV reservoir and are capable of passing HIV to cells of lymphoid and myeloid lineages [83]. HIV can also infect human bone marrow mesenchymal stem cells (MSCs), leading to impairment of MSC clonogenic activity [84]. Marrow MSCs are ancestor cells capable of generating heterologous stromal lineages including fibroblasts, osteoblasts and adipocytes. HIV nucleic acids can be readily detected within stromal colonies derived from bone marrow MSCs following exposure to HIV [84]. Microvascular endothelial cells in different organ tissues are permissive for HIV infection. Cultures of marrow stromal cells from HIV-seropositive patients have revealed that microvascular endothelial cells are predominant cells infected by HIV in the marrow stroma [85]. G-CSF production by marrow stromal cells of HIV-infected patients is significantly reduced.

A significant number of HIV-infected patients also exhibit bone loss with osteopenia and osteoporosis [86–88]. Live or heat-inactivated HIV virus and HIV gp120 protein induce apoptosis in human osteoblasts [89, 90]. Gp120 substantially inhibits human osteoblast cell proliferation [90]. In addition, osteocalcin synthesis in osteoblasts from HIV-infected patients is significantly reduced [91]. HIV is known to infect several types of mature hematopoietic cells including monocytes, macrophages and megakaryocytes [92–94].

HIV infection of these marrow stromal niche cell types may mediate myelosuppression and impairment of granulopoiesis through at least three mechanisms. First, HIV-infected niche cells serve as the immediate reservoir to produce infective viral particles and their protein components which, in turn, either infect or injure hematopoietic precursor cells residing in the marrow niche structures. Second, damage and/or loss of niche cells due to HIV cytotoxicity impair cell–cell contact between niche cells and hematopoietic cells,

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diminishing niche structural support to hematopoietic cells for myeloid/granulocyte lineage development. Third, infection with HIV and/or stimulation with HIV-derived components alter the production of cytokines and hematopoietic growth factors by niche cells, which compromises humoral regulation of myeloid/granulocyte lineage development in the bone marrow.

Several studies have tested the effects of HIV infection in marrow niche cells on the function of hematopoietic microenvironment. The adherent cell fraction or stromal cell monolayer is the *in vitro* equivalent of the *in vivo* bone marrow hematopoietic microenvironment [95, 96]. Monocytotropic HIV-1ADA infected human marrow stromal cell layers are unable to adequately support the CFU-GM activity of co-cultured CD34+ HSPCs in long-term bone marrow cultures [97]. Similar experiments examining the stromal cell monolayers in long-term bone marrow culture have shown that HIV infected marrow stromal cells cannot provide adequate support to normal CD34+ HSPC expansion and differentiation, as reflected by decreased CD34+ HSPC proliferation and colony forming activities [98]. In this culture system, there is no loss of hematopoietic support function when the stromal cell layers are resistant to HIV replication, either using murine stromal cell layers that are innately resistant to HIV infection or using human stromal cells genetically modified to express a gene that inhibits HIV replication (an RRE decoy).

Increasing evidence indicates that changes in soluble mediators in the marrow niche environment such as hematopoietic growth factors and cytokines affect myeloid/granulocyte development in patients with HIV infection [74, 80, 81]. Alteration of cytokine production by bone marrow stromal cells in HIV-infected subjects is characterized by decreased interleukin-2 (IL-2) and elevated tumor necrosis factor-alpha (TNF-α), macrophage inflammatory protein-1alpha (MIP-1α), MIP-1beta (MIP-1β) and regulated on activation normal T cell **e**xpressed and **s**ecreted (RANTES) levels. This alteration of the cytokine profile is associated with a decrease in marrow clonogenic activity [74, 80]. HIV Tat protein stimulates transforming growth factor-β1 (TGF-β1) production by human bone marrow macrophages [99]. HIV-infected patients frequently exhibit increased levels of circulating cytokines and chemokines including TNF-α, TGF-β, MIP-1β and RANTES [100–104]. TNF-α, MIP-1α and TGF-β are inhibitors of HSPC proliferation and myeloid differentiation [81, 99, 105–108]. Interleukin-3 (IL-3) and stem cell factor (SCF) are growth factors for HSPCs. IL-3 production by either whole blood mononuclear cells or isolated CD4+ T-cells is significantly decreased in patients with HIV infection [109]. The reduced IL-3 production in HIV-infected subjects is correlated with both the stage of the disease and signs of active viral replication. The serum level of SCF decreases during the course of HIV infection [110]. This decrease in serum SCF level correlates with a fall in the blood CD4+ T lymphocyte count. Granulocyte colony-stimulating factor (G-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF) are major lineage-specific growth factors for granulocyte lineage development. Cultures of marrow stromal cells from HIV-infected patients have shown that the stimulated release of G-CSF from these cells is significantly reduced [85]. In individuals without HIV infection, neutropenia can cause an increase in blood G-CSF level [111]. In HIV-infected patients, however, afebrile neutropenia is unable to stimulate G-CSF production in the circulation [112]. GM-CSF levels in the circulation of

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HIV-1 seropositive individuals are significantly lower than those of HIV-1 seronegative controls [113]. GM-CSF production by blood mononuclear cells and T-lymphocytes declines progressively following HIV infection [114, 115]. The decline in GM-CSF production is clearly correlated with the viral load in patients' peripheral blood mononuclear cells [114]. This impairment of GM-CSF production is especially profound in the advanced stage of HIV disease. The reduction of GM-CSF production correlates with the decrease in the number of circulating CFU-GM cells in patients with HIV infection. *In vitro* experiments utilizing replication incompetent HIV-1 mutants have revealed that deletion of Vpr abrogates the marrow stromal cell defect in producing granulopoietic growth factors [116]. The inhibition of G-CSF expression by HIV Vpr has also been observed in cultures of bone marrow stromal cells transduced with the Moloney-based expression vector containing *vpr*. These observed derangements of hematopoietic growth factor and inflammatory cytokine profiles in the marrow niche environment and systemic circulation are clearly not favorable for maintaining normal hematopoietic activity and myeloid/granulocyte lineage development in the bone marrow following HIV infection.

HIV infection associated autoimmune disorders—Polyclonal B-cell activation and generation of antineutrophil autoantibodies are common clinical findings of HIV disease [117–119]. Patients with HIV infection who developed an infectious mononucleosis-like episode with neutropenia and thrombocytopenia exhibit antigranulocyte antibodies in their serum samples and granulocyte eluates [118]. Specific antineutrophil autoantibody (anti-MAC-1) activity has been reported to exist in as many as 45% of individuals with HIV infection, which is highly correlated with the development of neutropenia [117]. In addition to antibodies against neutrophil surface membrane antigens, immunoglobulins against neutrophil cytoplasmic molecules including myeloperoxidase and elastase have also been detected in HIV-infected patients [119]. Examinations of serum samples from 105 HIV-infected patients have shown that antineutrophil cytoplasmic antibodies are positive in 45 of them [120]. At the present time, however, the clinical significance of these antineutrophil cytoplasmic antibodies is unclear. Studies have not yet confirmed any correlation of detected anti-neutrophil cytoplasmic antibodies with the alteration of immunological status, the presence of an opportunistic infection, the development of malignancy, or the survival of patients with HIV infection [119, 120].

Complication with opportunistic infections—In patients with HIV disease, particularly those in the advanced disease stage, complication with opportunistic infections can induce or exacerbate myelosuppression and neutropenia [6, 121–123]. Certain obligate intracellular microbes cause myelosuppression by direct invasion of the bone marrow. Mycobacterial infections including those caused by *Mycobacterium avium* complex and *Mycobacterium tuberculosis* frequently involve the bone marrow [124–130]. Examinations of bone marrow samples have shown that infection with *Mycobacterium avium-intracellulare* complex exists in 20% to 24% of patients with HIV infection and/or AIDS [126, 127]. A study of 56 adult patients with HIV disease who underwent a single bone marrow aspiration, biopsy, and culture because of unexplained fever and/or other clinical features suggestive of mycobacterial infection has reported that 32 patients (57%) are

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ultimately diagnosed with *Mycobacteriumavium* complex/*Mycobacteriumtuberculosis* or *Histoplasmacapsulatum* infection [128].

Infection with opportunistic fungal pathogens including *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Coccidioides immitis, Candida albicans* and *Pneumocystis carinii* has also been implicated in bone marrow injury and myelosuppression [33, 122, 125, 131, 132]. Myelosuppression associated with these opportunistic fungal infections most frequently results from direct involvement of the bone marrow during infection. *Pneumocystis carinii* is an extracellular fungal pathogen. Pneumocystis infection promotes osteoclastogenesis along with bone marrow failure through disruption of type I interferon signaling and induction of osteoclastogenic factors, including the receptor-activated nuclear factor-κB ligand and the proapoptotic factor tumor necrosis factor-related apoptosis-inducing ligand, in conjunction with their shared decoy receptor osteoprotegerinas [133, 134].

Secondary infections with viral pathogens such as *cytomegalovirus* (CMV) and hepatitis B virus (HBV) are frequent in HIV-infected patients [135, 136]. In some cases, these secondary viral infections are severe. CMV infection can cause or aggravate neutropenia [137, 138]. CMV infects both HSPCs and stromal cells, so that the entire haematopoietic system is a target for CMV dissemination and latency [139]. As a result, myelosuppression can result from both direct inhibition of HSPC growth and indirect impairment of the marrow stromal cell support for HSPC commitment to myeloid lineage development [139–143]. Although the primary site of HBV pathogenic impact occurs in the liver, involvement of bone marrow hematopoietic cells and stromal cells as well as peripheral blood granulocytes has been observed during HBV infection [144–146]. Hepatitis B surface antigen (HBsAg) can be frequently detected in the nuclei of immature hematopoietic cells including myeloblasts during HBV infection, which demonstrates the close interaction between HBV and marrow hematopoietic precursor cells. HBV infection often causes hematopoietic suppression. *In vitro* exposure of human bone marrow cells to HBV-containing sera results in a dose-dependent inhibition of HSC myelopoietic (CFU-GM) activity [144]. Inactivation or immunoabsorption of HBV from the sera abolishes this inhibition of HSCs, indicating that the HBV virion is responsible for this suppression of hematopoietic cell function.

Protozoal infection such as toxoplasmosis in HIV-infected patients has been reported to involve the bone marrow [147–149]. Bone marrow examinations of HIV-infected patients with pancytopenia have demonstrated *Toxoplasma gondii* within the cytoplasm of marrow cells belonging to myeloid lineages including macrophages, granulocytes and megakaryocytes [149, 150].

Certain secondary infections with bacterial pathogens can also cause myelosuppression and neutropenia in HIV-infected individuals. Patients with HIV infection have a significantly increased risk of *Salmonella* infection [151, 152]. Salmonellosis in HIV-infected patients is unusually severe, characterized by widespread infection involving multiple organ systems, bacteremia and sepsis [151, 153]. Salmonellosis causes neutropenia by infecting marrow hematopoietic cells and suppressing granulocyte development [154].

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In addition to marrow toxicity and myelosuppression, increased peripheral destruction of granulocytes also contributes to neutropenia. HIV-infected patients with secondary infections caused by different pathogens including mycobacteria, fungi, CMV, *Toxoplasma gondii* and *Salmonella* can present with splenomegaly [155–157], which may lead to an increase in peripheral destruction of granulocytes along with other blood cell types such as erythrocytes and platelets.

Hemophagocytic syndrome—An uncommon complication of HIV infection known as the hemophagocytic syndrome (or hemophagocytic lymphohistiocytosis) is characterized by proliferation of histiocytes and phagocytosis of marrow hematopoietic precursors. Pancytopenia including neutropenia and splenomegaly are among its typical manifestations. Viral infection with or without other disorders may impair the activities of NK cells and cytotoxic T-cells, leading to cytokine deregulation with proliferation and activation of histiocytes. HIV infection itself can cause this syndrome [158–161]. Hemophagocytic syndrome may also occur in HIV-infected patients in association with diverse secondary infections and malignancies, including infection with *Epstein-Barr virus*, CMV, *herpes simplex virus*, *Kaposi's sarcoma-associated herpesvirus*, *parvovirus B19*, *Mycobacterium tuberculosis*, *Pneumocystis carinii, Toxoplasma gondii or Candida albicans* and malignancies such as Hodgkin's lymphoma, non-Hodgkin's lymphoma, and Kaposi's sarcoma [162–171].

Use of therapeutic agents with myelotoxicity—A number of agents used to treat HIV and opportunistic infections can cause hematological abnormalities. Among agents used for treatment of primary HIV infection, nucleoside reverse transcriptase inhibitor azidothymidine (zidovudine or AZT), non-nucleoside reverse transcriptase inhibitor delavirfine (DLV), protease inhibitors (PIs) ritonavir (RTV) and nelfinavir (NFV) have a side effect of causing neutropenia [123,172]. Medications for secondary infections in HIV-infected individuals including antiviral agents such as ganciclovir, cidofovir, foscarnet and pegylated interferon, antifungal drugs such as amphotericin B and pentamidine, as well as antibacterial remedies such as rifabutin, trimethoprim-sulfamethoxazole and dapsone exhibit bone marrow toxicity [123, 173, 174]. In addition, certain chemotherapy agents used to treat malignancies associated with HIV infection are marrow toxic and can cause various extents of neutropenia.

Association with confounding factors—HIV-infected individuals often display confounding factors, particularly excessive alcohol consumption and drug abuse. One prospective cohort study has reported that 82% of HIV infected patients consume alcohol, and 41% have Michigan Alcoholism Screening Test (MAST) scores classifying them as alcoholics [175]. In a study of 220 HIV-infected drug users, 63% of them are found heavily consuming alcohol [176]. Another analysis of alcohol consumption in 866 HIV-infected veterans has shown that 247 (29%) are past drinkers and 577 (67%) are current alcohol users [177]. Among the 824 reporting past or current alcohol use, 192 (23%) drink hazardously, and 291 (35%) carry a diagnosis of alcohol abuse or dependence. Hazardous drinkers more often have detectable viral loads [178]. This clinical feature is supported by observations from experimental studies that excessive alcohol exposure enhances HIV replication in

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human peripheral blood mononuclear cells [179, 180]. Alcohol feeding also results in a significant elevation of the viral load in the systemic circulation of nonhuman primates infected with SIV or SIV/HIV [181–185]. The level of alcohol consumption has a linear association with bacterial pneumonia, candidiasis and co-infection with hepatitis C virus in HIV-infected patients [177]. Hazardous alcohol consumption accelerates HIV disease progression in human subjects [177, 178] and SIV disease progression in nonhuman primates [182]. Alcohol abuse may promote HIV disease progression through activating replication of HIV virus, aggravating the loss of circulating CD4+ T cells, and increasing the incidence of secondary infections with opportunistic pathogens [176, 179, 180, 186–189], all of which may potentially exaggerate the development of neutropenia in individuals infected with HIV.

Excessive alcohol consumption is hazardous to hematopoietic tissue [190–194]. Bone marrow samples from alcohol-abusing individuals exhibit a significant reduction in the number of mature granulocytes and vacuolization of myeloid progenitor cells [195–197]. Exposure of bone marrow cells to alcohol at concentrations commonly observed in intoxicated patients suppresses granulocyte colony formation [195, 198]. In experimental animals, alcohol intoxication injures primitive hematopoietic precursor cells and inhibits HSPC proliferation as well as their commitment to myeloid/granulocyte lineage development by disrupting fundamental cell signaling regulation of granulopoietic cell function [199–202]. The ERK-cyclin D1 pathway plays an important role in mediating HSPC proliferation [203]. Alcohol exposure suppresses the activity of ERK-cyclin D1 signaling in HSPCs [204]. As described above, the HIV protein gp120 is cytotoxic to HSPCs and marrow hematopoietic niche cells. Gp120 is degraded through the lysosomal/ubiquitin system. Chronic alcohol consumption impairs lysosomal/ubiquitin-mediated degradation of intracellular gp120 and the release of gp120 fragments [205]. This alcohol-induced impairment of gp120 decomposition potentiates its marrow cytotoxicity. Alcohol induces a profound inhibition of myeloid progenitor cell proliferative activity in hosts carrying the HIV-1 *tat* gene product [206]. Alcohol can also exert direct damages to the marrow hematopoietic niche microenvironment [190, 195, 198, 207–210]. Alcohol abuse is known to cause osteoporosis and osteonecrosis [207]. Chronic alcohol consumption causes a significant change in hormonal and growth factor homeostasis in the body, which promotes adipogenesis and triglyceride accumulation in the bone marrow [207–209]. When marrow MSCs are exposed to ethanol, there is a shift from osteogenic to adipogenic preference of differentiation [209, 211–213]. Adipocyte differentiation is less capable of supporting myelopoietic lineage cells [214]. In addition, replacement of hematopoietic tissue (red marrow) by adipose tissue (yellow marrow) as a result of the increased adipogenic activity in the bone marrow reduces the overall capacity of hematopoiesis in hosts who excessively consume alcohol. In the clinic, alcohol abusers with severe bacterial infections frequently present with granulocytopenia, which is an indicator of increased mortality [215]. In addition to the direct bone marrow toxicity, alcohol intoxication suppresses granulocyte functional activities including chemotaxis, phagocytosis, intracellular killing of ingested pathogens, and the production of antimicrobe molecules [216–218].

Many other frequently abused drugs, including opioids, cocaine, cannabinoids and methamphetamine, have been implicated as risk factors for HIV infection, as they all have

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the potential to compromise host immunity and facilitate viral replication [219, 220]. Available data from large cohort studies of HIV-infected individuals in the United States and in Europe present mixed results regarding the effects of drug abuse on HIV disease progression [219]. It can be assumed that the abuse of any agent that compromises host immunity and/or facilitates HIV replication in the infected host would consequently exert a negative impact on marrow hematopoietic activity due to HIV cytotoxicity. At the present time, however, information about the link between abuse of illicit drugs and the development of neutropenia in patients with HIV infection remains unclear. One fact deserving special attention is that HAART, the best known regimen effective for limiting HIV disease progression, is often withheld from injection drug users (IDUs) with HIV infection based on the belief that their unstable lifestyle may predetermine a markedly inferior outcome with HAART and an increased risk of developing drug resistant viral strains [221, 222]. If myelosuppression and neutropenia develop in IDUs infected with HIV, these patients are in a very unfavorable situation due to the reduced availability of optimal medical care for the prevention and treatment of their disease.

CURRENT STATUS OF TREATMENT

Since multiple factors can cause neutropenia during HIV infection, treatment of this health problem relies on identifying the cause(s) as well as delineating the underlying mechanism(s). Specific management may vary from case to case, depending on the etiology and severity of neutropenia. A mild decrease in neutrophil count in an asymptomatic patient may need no specific management except for closely monitoring disease development. However, severe neutropenia needs to be appropriately treated in order for the HIV-infected individual to avoid fatal secondary infections.

Antiretroviral therapy

Severe neutropenia frequently occurs in association with the substantial loss of CD4+ T cells and the increase in HIV viral load in advanced HIV disease [21, 223, 224]. Therefore, effective treatment of primary HIV infection to control the disease progression is critically important for both the prevention and treatment of neutropenia in patients infected with HIV. NIH guidelines for antiretroviral therapy (ART) in HIV-infected adults and adolescents suggest that treatment should be offered to all patients with symptoms ascribed to HIV infection [225]. Recommendation for ART among asymptomatic patients requires analysis of real and/or potential risks and benefits. In general, treatment should be offered to persons who have <350 CD4+ T cells/μL or plasma HIV ribonucleic acid (RNA) levels of >55,000 copies/mL [by b-deoxyribonucleic acid (bDNA) or reverse transcriptase-polymerase chain reaction (RT-PCR) assays] [225]. In support of active ART, an analysis of 24 444 patients who were followed from the beginning of treatment has shown that deferring antiretroviral combination therapy until the CD4+ cell count drops to 251–350 cells/μL is associated with higher rates of AIDS and death than starting therapy when the cell count is within 351–450 cells/μL [226]. The adverse effects of deferring treatment increase with the decreased CD4+ cell count threshold, indicating that 350 CD4+ cells/μL should be the minimum threshold count for initiation of ART. A recent investigation on the effect of HAART on neutrophil counts in 1729 HIV-infected women has demonstrated that worse HIV disease parameters,

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such as lower CD4+ cell counts and higher HIV-1 RNA levels, are more strongly associated with development of neutropenia [21]. Treatment with HAART, without AZT in the regimen, protects against the development of neutropenia. Resolution of neutropenia can be achieved with HAART treatment and an increase in CD4+ cell counts. Compared with patients without any ART, HIV-positive patients receiving combination ART without AZT and even those receiving HAART with AZT are more likely to resolve their neutropenia. Since HAART treatment can attenuate or reverse neutropenia in most HIV-infected patients (91.1%), control of HIV infection with HAART is critically important in the management of hematological manifestations in patients with HIV disease [227]. A comparison of patients treated before and after the introduction of HAART has identified neutropenia as an independent risk factor for bacteremia. Introduction of HAART treatment significantly reduces the incidence of neutropenia and episodes of bacteremia in patients infected with HIV [40].

It is well known that AZT and certain other antiretroviral compounds have marrow toxicity and induce myelosuppression. During the treatment of neutropenia in HIV-infected patients, health care providers should consider the hematological side effects of these agents. A meta-analysis of the impact of AZT versus stavudine (d4T) in HAART triple therapy regimens on treatment of HIV-infected patients with regard to hematologic parameters and efficacy markers has shown that treatment efficacy, as measured by changes in CD4+ T cell counts and viral load, does not differ significantly between regimens [228]. However, AZT-based HAART has a greater negative impact on hematologic parameters relative to d4T-based regimens. AZT recipients are more likely than d4T recipients to experience anemia and neutropenia events of any grade. When patients develop severe neutropenia during the course of AZT-based HAART treatment, switching to other HAART regimens without AZT may be advised. In some cases, switching HAART regimens during the treatment of HIV infection can be challenging. This regimen switch requires considerations of a thorough drug treatment history and the results of drug resistance testing. An optimal change in therapy should achieve maximal and durable suppression of the viral load, as well as restoration and preservation of immunologic function, improving quality of life and reducing HIV-related morbidity and mortality [225].

Treatment of complications and elimination of confounding factors

Bone marrow suppression and neutropenia associated with or exaggerated by secondary infections and malignancies in HIV-infected patients require specific treatments for the corresponding complications. Other confounding factors, including abuse of alcohol and illicit drugs, should be identified in HIV-infected individuals with neutropenia. Assistance for adjusting life style to discontinue substance abuse will clearly benefit the treatment of disease.

Application of hematopoietic growth factors

Clinical and experimental studies have indicated that the application of hematopoietic growth factors including G-CSF and GM-CSF may be helpful in overcoming the myelosuppression induced by HIV or by many therapeutic agents. Native human G-CSF is a 22-kD glycoprotein that selectively stimulates the proliferation and maturation of myeloid

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progenitor cells to granulocytes [216, 217]. Cells at all stages of the granulocyte lineage, including their up-stream precursors (marrow CD34+ CD33− cells), have G-CSF receptors (G-CSFR) and respond to G-CSF with increased proliferation. This cytokine plays a critical role in maintaining the normal blood level of granulocytes and is responsible for increasing the number of circulating neutrophils during infection and inflammation. G-CSF also enhances the functional activities of neutrophils including adhesion molecule expression, chemotaxis, oxygen metabolism, phagocytosis and intracellular bacterial killing. Treatment with recombinant human methionyl G-CSF (filgrastim) can alleviate neutropenia in patients with HIV infection [6, 229]. A multicenter study of 200 HIV-infected patients with neutropenia has shown that filgrastim reverses neutropenia in 98% of patients, with a median time to reversal of 2 days (range 1–16 days) [230]. In this cohort, 83% of patients have received one or more therapeutic agents that are most frequently considered to cause neutropenia. Filgrastim allows $>80\%$ of patients to increase or maintain dose-levels of these medications, or even add such medications to their therapy. In addition, filgrastim is well tolerated by patients without a negative effect on HIV-1 p24 antigen levels. Accumulated evidence indicates that filgrastim treatment can provide an immediate and sustained increase in blood neutrophil counts and significantly decreases the incidence of severe neutropenia, bacterial infection, or death [231, 232].

G-CSF is a lineage-specific growth factor that selectively promotes myeloid differentiation and granulocyte production in the bone marrow. Information about the effect of G-CSF therapy on the size of HSC pool as well as the self-renewal capacity of these primitive hematopoietic precursors remains limited. Exogenous G-CSF enhances bone marrow release of HSPCs into the systemic circulation [233, 234]. Patients with advanced HIV disease (<500 CD4+ cells/μL blood) are less able to mobilize CD34+ cells from the bone marrow in response to G-CSF administration [76], suggesting a possibly diminished bone marrow reserve of HSPCs. It is unknown whether G-CSF-induced enhancement of HSC commitment to granulocyte development and mobilization of HSPCs out of the bone marrow would add to the depletion of HSCs in hosts with advanced HIV disease. One randomized, placebo-controlled trial has examined the long-term effect of G-CSF on absolute numbers of CD34+ cells and progenitor cell function in HIV-infected patients [235]. Administration of filgrastim (300 μg) three times weekly for 12 weeks increases the number of CD34+ cells and CFU forming activity in the systemic circulation of HIV-infected patients. This alteration of CD34+ cell number and CFU activity in the circulation is associated with increases in total white-blood cell and CD4+ cell counts. In the meantime, blood hemoglobin and platelet counts are decreased.

GM-CSF is a 23-kDa secreted polypeptide that functions as a growth factor, predominantly stimulating bone marrow cells of myeloid lineages. Compared to G-CSF, GM-CSF has a broader spectrum of myelopoietic stimulation that includes the development of granulocytes, monocytes, macrophages, dendritic cells, erythrocytes and megakaryocytes [217]. GM-CSF also modulates functional activities of monocytes and macrophages, including Fcγ receptor expression, antibody- and complement-mediated phagocytosis, antigen presentation, cytokine production, oxygen metabolism and intracellular killing of microbes. A clinical study on recombinant human GM-CSF treatment of leucopenic patients with HIV infection has reported that administration of GM-CSF results in a significant increase in white blood

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cell counts [236]. Neutrophils, eosinophils and monocytes are responsible for most of the increase in white blood cell counts. Mild flu-like side-effects occur in most patients receiving GM-CSF, but they are generally not severe to warrant withdrawal from the treatment. A phase III clinical trial of GM-CSF treatment in HIV-infected patients has also demonstrated significant increases in both neutrophil and CD4+ cell counts in the GM-CSF group [237]. GM-CSF treatment significantly reduces the incidence of overall infections and delays the time to first secondary infection in HIV-infected patients.

The effects of GM-CSF therapy on HIV virological parameters remain controversial. *In vitro*, GM-CSF enhances HIV-1 replication in bone marrow CD34+ cells during monocyte/macrophage differentiation [49], in monocyte-derived macrophages cultured at low density [238], and in cultured human brain tissues [239]. A clinical trial has reported that GM-CSF induces a modest increase in plasma HIV-1 RNA levels in patients with uncontrolled HIV infection [240]. In contrast, another clinical study has shown that GM-CSF administration does not affect virological parameters in patients with HIV infection [241]. Furthermore, some *in vitro* studies have documented that GM-CSF consistently suppresses HIV replication in human monocyte-derived macrophages [242]. This inhibitory effect of GM-CSF on HIV-1 replication can be observed regardless of the HIV-1 strain, source of GM-CSF, stage of macrophage maturation or timing of GM-CSF exposure in relation to HIV-1 infection. The inhibitory effect of GM-CSF is dose-dependent and can be reversed by neutralizing antibodies. Evaluation of GM-CSF effect on viral load and CD4 cell count during interruption of HAART in HIV-infected patients has found that viremia is approximately two to three times lower in the group receiving GM-CSF. The majority of patients in the scheduled treatment interruption group exhibit a decrease in their CD4+ cell counts. Such decreased CD4+ cell counts generally do not occur in patients treated with GM-CSF [243]. The reasons for these discrepant findings still wait for clarification. Further investigations on large cohort patients will provide precise information regarding the impact of GM-CSF treatment on virological features in patients with HIV infection.

In addition to myeloid lineage specific growth factors, the effects of early-acting hematopoietic growth factors including SCF and IL-3 on the treatment of myelosuppression associated with HIV infection have also been explored. SCF is a multipotential growth factor acting on HSCs and early progenitor cells of most hematopoietic lineages [244, 245]. Serum levels of SCF are increased in asymptomatic HIV-infected patients [110]. With HIV disease progression, the serum level of SCF decreases. A Cox proportional hazards model shows SCF to be an independent prognostic factor for survival. IL-3 functions on earlier stages of lineage commitment, regulating the proliferation and expansion of primitive hematopoietic progenitors including early myeloid progenitor cells. [244, 246, 247]. *In vitro* studies suggest that SCF or IL-3 may have a therapeutic application in overcoming hematopoietic abnormalities, particularly the myelosuppression associated with drugs commonly used in AIDS patients [107]. In SIV-infected non-human primates, IL-3 administration increases peripheral blood CD34+ cells and total CFUs without changing the viral load [248]. Currently, information about the direct clinical application of these early-acting hematopoietic factors for the treatment of myelosuppression and neutropenia in patients with HIV infection remains sparse.

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HSC gene engineering

Reconstitution of bone marrow and host immune system using HSC gene engineering represents a novel direction for the treatment of HIV infection and associated hematological abnormalities [249–251]. It has been reported that the viral replication in an HIV-infected patient with relapsed acute myelogenous leukemia is effectively controlled after transplantation of HSCs from a donor with the deletion of the HIV co-receptor CCR5 gene (CCR5 32 homozygous donor) [252]. This accomplishment suggests that HSCs can be modified so that they and their progeny would be able to resist HIV infection [250, 251, 253, 254]. After introduction of these modified HSCs, the host could be repopulated with an HIV-resistant hematopoietic system, including CD4+ T cells and myeloid targets. To achieve this goal, multiple cutting-edge technologies need to be developed and/or refined for each step of the therapeutic procedure, including obtaining sufficient numbers of highly purified long-term repopulating HSCs from the donor, effective gene engineering of HSCs for HIV resistance without any hazardous potential of developing malignancy or hematological defects, stable expansion of gene-engineered HSCs *in vitro*, safely implanting HIV-resistant HSCs and flourishing engraftment of implanted HSCs in the recipient, successful replacement of marrow hematopoiesis and reconstitution of a HIV-resistant immune system in the HIV-infected host, and eventually curing HIV infection as well as associated hematological abnormalities.

At the present time, efforts in developing HSC-based gene therapy for treatment of HIV infection and the associated hematological derangements remain in the preclinical stage. Various gene engineering targets have been explored within two major categories: disrupting cellular genes that facilitate HIV entry (such as CCR5 and CXCR4 coreceptors) and introducing genes that interfere with HIV replication [fusion inhibitors such as gp41-derived peptide (C46); genome disruption such as evolved recombinases, endonucleases; gene expression inhibitors such as TAR decoys, anti-Tat ribozymes; RNA export inhibitors such as dominant mutant or transdominant forms of Rev; or host restriction factors such as TRIM5a, APOBECs and tetherin] [250, 251]. In addition to the efficacy and safety of gene manipulation in each step of the therapeutic procedure described above, another potential obstacle to this approach is that HIV infects various marrow niche cell types from origins other than HSCs. It remains unclear whether and how the disrupted hematopoietic niche environment in individuals infected with HIV would be able to support the successful reconstitution of the hematopoietic/immune system through genetically engineered HIV-resistant HSCs.

CONCLUSION

Neutropenia and myelosuppression frequently occur in individuals infected with HIV. Transient and moderate reduction of blood neutrophils in asymptomatic patients with HIV infection may not need specific medical intervention. Severe neutropenia, particularly when it occurs in patients with advanced HIV disease, is an independent risk factor for developing fatal secondary infections. The etiology of neutropenia during HIV infection is multi-factorial, which may include HIV cytotoxicity, complications of secondary infection and malignancy, and the effects of myelosuppresive agents used for treatment. Precise diagnosis

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with a clear understanding of mechanisms responsible for the neutropenia is critical for providing specific treatment. In most cases, severe neutropenia is associated with the significant loss of CD4+ cells and an increase in viral load in patients infected with HIV. Appropriate HAART treatment is beneficial for reversing myelosuppression and neutropenia. Adjuvant treatment with granulocyte lineage specific growth factors, particularly G-CSF, is helpful for reversing neutropenia and controlling serious complications of bacterial infection. Confounding factors causing bone marrow injury, such as the abuse of alcohol and other drugs, should be eliminated in neutropenic patients with HIV infection. Efforts in exploring innovative approaches for treatment of HIV disease including gene engineering based HSC therapy to reconstitute a HIV-resistant hematopoietic/immune system may contribute to the improvement of medical care for patients suffering from HIV infection and the associated hematological abnormalities in the future.

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TABLE 1

Causes of neutropenia in HIV infection.

Cause	Potential mechanism
HIV toxicity to HSPCs	HIV infection and replication in HSPCs Cytotoxicity of HIV proteins including gp120, Nef, Gag p24 and Tat
Impairment of hematopoietic niche	HIV infection of niche cells Local production of HIV by infected niche cells Impairment of niche cell-cell contact support to hematopoiesis Disruption of hematopoietic growth factor and cytokine environment in the niche network
Autoimmune disorders	Generation of anti-neutrophil autoantibodies
Marrow toxicity of secondary infections	Invasion of the bone marrow by pathogens responsible for secondary infections Increase in peripheral destruction of granulocytes
Hemophagocytic syndrome	Activation of histiocytes and phagocytosis of hematopoietic precursor cells
Therapeutic agents with myelotoxicity	Cytotoxicity to HSPCs
Confounding factors such as alcohol abuse	Cytotoxicity to HSPCs Disruption of hematopoietic niche environment