

The Role of Hematopoietic Progenitor Cells in Retroviral Pathogenesis

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INTRODUCTION

Hematopoiesis is the process by which hematopoietic stem cells (HSCs) give rise to all the cellular components of the hematopoietic system. HSCs have the potential to proliferate indefinitely, and can differentiate into mature hematopoietic lineage-specific cells. The bone marrow is the principal site for hematopoiesis in adults. HSCs proliferate within the bone marrow and differentiate to produce the requisite number of highly specialized cells of the hematopoietic system. HSCs give rise to two different populations of cells: (1) a common lymphoid progenitor (CLP) population, that generates B cells, T cells and NK lineage cells; and (2) a common myeloid progenitor (CMP) population that generates granulocytes, neutrophils, eosinophils, macrophages and erythrocytes. Lineage commitment by HSCs is a complex process that can be induced in response to a variety of factors, including

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relative levels of hematopoiesis associated cytokines and transcription factors. These factors not only help maintain the pluripotency of HSCs but can also actively induce commitment and differentiation of HSCs.¹⁻¹⁰ By virtue of their importance in cell proliferation and differentiation, any deregulations in the cytokine milieu or modulation of hematopoiesis-associated transcriptional factors may result in the disruption of cell cycle or lineage commitment of these cells leading to perturbation of hematopoiesis.

One of the major causes of disruption of this hematopoietic network is viral infection.¹¹ Viruses such as human immunodeficiency virus-1 (HIV-1), human cytomegalovirus (CMV) and human herpes virus-8 (HHV-8) have been previously shown to disrupt the normal pattern of hematopoiesis either by infecting bone marrow resident stromal cells or by direct infection of hematopoietic progenitor cells which can result in alteration in the cytokine milieu/transcription factor levels and subsequent perturbation of hematopoiesis.¹²⁻¹⁴ Viral infection within the bone marrow results not only in the perturbation of hematopoiesis, but infected HSCs may also become a source for the generation of infected lineage-specific cells that may differentiate into various hematologic malignancies. Retroviruses can establish latent infection in HPCs (hematopoietic progenitor cells) and HSCs, resulting in perturbation of hematopoiesis and induction of viral pathogenesis.

VIRAL INFECTION IN HP/HSCs

A primary infection of HSCs with virus requires the expression of corresponding cellular receptors for binding and internalization of that particular virus. It has now been well established that HP/HSCs express cellular receptors and as a result they become susceptible to various viral infections. However, since destruction of infected cells by various effector cells of the immune system leads to the clearance of infection, viruses have evolved elaborate strategies to evade the immune system and thus to persist within the host. One of the most prominent mechanisms of viral evasion is through the infection of progenitor cells within the bone marrow, which is a relatively immune-privileged site. Viral infections in HSCs can have deleterious effects, including induction of cytolytic and apoptotic cell death resulting in the suppression of hematopoiesis as well

as dysregulation of normal development of HSCs, leading to subsequent outgrowth of malignant cells pertaining to a particular hematopoietic lineage. The marrow microenvironment is a complex system comprising many cell types, including stromal cells that produce cytokine/growth factors as well as adhesion molecules that are vital for the maintenance, differentiation and maturation of HP/HSCs.^{1,3} Infection of bone marrow stromal cells can make these cells incapable of supporting hematopoiesis, resulting in multilineage hematopoietic failure.¹⁵ To this end a variety of viruses have clinically important effects on the hematopoietic system. CD34⁺ HPCs have been shown to be susceptible to infection with a number of viruses: HIV-1, hepatitis C virus, JC virus, Parvovirus, HCMV, HHV-5, HHV-6, HHV-7, HHV-8 and HTLV-1^{12,16–28} (Fig. 1). Suppression of hematopoiesis has been documented to occur following infection of CD34⁺ HPCs with HCMV/HHV-5, HHV-6, HIV-1, and measles virus either by direct infection of CD34⁺ HPCs or by indirect mechanisms, such

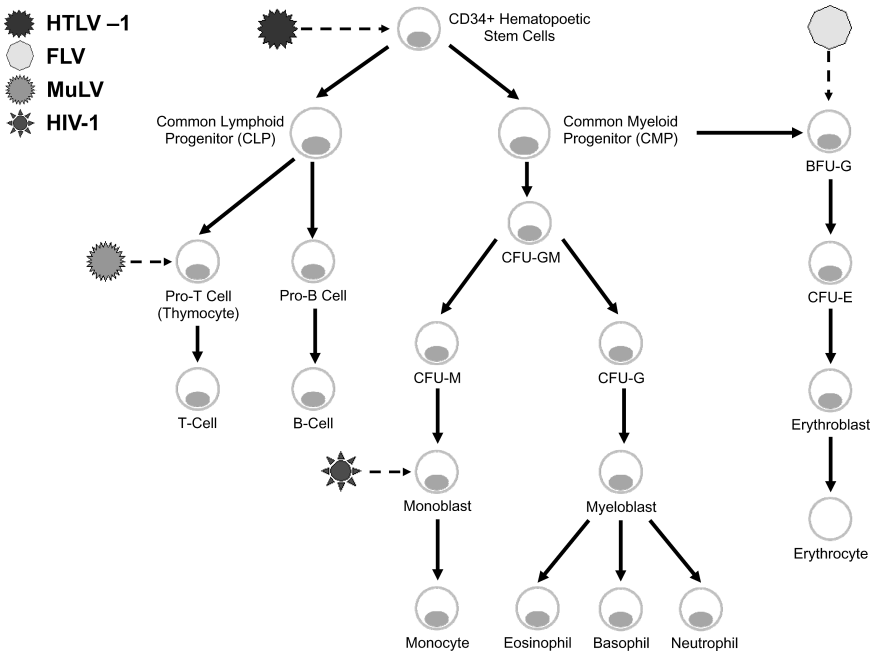


Fig. 1. Susceptibility of progenitor cells in the hematopoietic system to retroviral infection.

as the disruption of cytokine expression via infection of bone marrow stromal cells. The authors' laboratory has reported that HTLV-1 and KSHV infection of CD34⁺ HPCs results in suppression of hematopoiesis both *in vitro* and *in vivo* in humanized SCID mice.^{28–30} The range of effects resulting from retroviral infection of hematopoietic progenitor cells leading to dysregulation of hematopoiesis and manifestation of various hematological malignancies are discussed in this chapter.

RETROVIRUSES AND CANCER: SIGNIFICANCE OF INFECTION OF HEMATOPOIETIC PROGENITOR CELLS

The first association of retroviruses with malignancies was reported with the observation that spontaneous erythroleukemia in chickens was infectious, which was later shown to be associated with avian erythroblastosis virus (AEV) infection.³¹ In 1911, Peyton Rous showed that some avian sarcomas could be transmitted through inoculation of cell-free extracts, proving for the first time that viruses can be linked to cancer. However, these early studies were largely ignored, and it was not until observations made by Bittner and Gross in mice with mammary carcinoma and thymic lymphoma that these malignancies resulted from viral infections, that an association between cancers and retroviral infections was firmly established.^{32–34} Since the 1960s, retroviruses have been shown to cause leukemia, lymphoma and other forms of cancer in a wide variety of vertebrate animals, ranging from fish to humans. The first oncogenic human retrovirus, human T cell leukemia virus-type 1 (HTLV-1) was discovered in 1980 and has subsequently been associated with the development of adult T-cell leukemia/lymphoma (ATL).^{35,36} HIV-1 was discovered and characterized a few years later and the study of both animal and human retroviruses has led to the fundamental understanding of the multistep process of oncogenesis and has provided insights into complex human biological processes such as hematopoiesis, since retroviruses often target cells of the hematopoietic system. In this chapter, we will discuss the effects of retroviral infection on hematopoietic progenitor cells and their role in the development of malignancies. During the process of hematopoiesis, HSCs commit to decide between the self-renewal and the differentiation/maturation pathways to give rise

to mature lineage-committed hematopoietic cells. Under normal conditions the ability of the HSC to undergo self-renewal is irreversibly lost, but in leukemia, progenitor cells can undergo unlimited self-renewal without entering the differentiation pathway.³⁷ On the contrary, dysregulation of hematopoiesis-associated transcription factors and cytokines can lead to cell-cycle arrest and induction of apoptosis in HSCs. Retroviruses have evolved a variety of mechanisms to both induce continuous self-renewal of infected hematopoietic progenitor cells as well as suppression of hematopoiesis by induction of cell cycle arrest or apoptosis in hematopoietic progenitor cells.

RETROVIRUS INDUCED LEUKEMOGENESIS

Avian Erythroblastosis Virus (AEV)

Avian erythroblastosis virus (AEV), which was isolated from a spontaneous erythroleukemia from chickens in 1908, was the first retrovirus discovered and has subsequently been associated with cancer.³¹ AEV is an acute leukemogenic retrovirus that belongs to the alpha retrovirus family and causes fatal erythroleukemia when injected into the ova or in young chickens.³⁸ Studies done *in vitro* indicate that development of malignancy is associated with co-infection of both AEV and a helper virus and that the target cell for infection is a committed erythroid progenitor cell, the burst forming units-erythroid (BFU-E), which becomes terminally blocked for further differentiation following infection.^{39,40} AEV encodes for two viral oncoproteins, *v-Erb-A*, which represents a mutated, oncogenic thyroid hormone receptor α (c-Erb-A/TR α), and *v-Erb-B*, which is a mutated constitutively active transmembrane receptor for epidermal growth factor (EGF).^{41,42} The AEV oncoprotein *v-Erb-A*, unless expressed at high levels, has no transformation capacity on its own but the co-expression of both *v-Erb-A* and *v-Erb-B*, leads to a severe leukemic phenotype.⁴³⁻⁴⁸ It is currently believed that *v-Erb-A* actively cooperates both with ligand activated receptor tyrosine kinases such as stem-cell factor (SCF) activated c-kit as well as constitutively activated viral oncoprotein *v-Erb-B*, to arrest differentiation and induce leukemia in erythroid progenitor cells.^{48,49} The binding of the thyroid hormone (T3) to the thyroid hormone receptor

(TR α) leads to the transcriptional activation of a multitude of target genes, some of which are required for normal erythropoiesis.⁵⁰ Since *v-Erb-A* is a mutant form of TR α that retains the DNA binding capability of the receptors but cannot bind to its ligand, T3, it has been suggested that *v-Erb-A* mimics TR α which, in the absence of the ligand, induces transcriptional repression of a host of target genes including those involved in differentiation of erythropoietic progenitors.⁵¹ The process involves c-Kit as well as downstream signal transducers, including the other viral oncoprotein *v-Erb-B* which has been shown to induce proliferation and self-renewal of erythroid progenitors. Ultimately, this leads to long-term proliferation and a block in differentiation of primary erythroid progenitor cells.⁵²

Murine Leukemia Virus

The discovery and characterization of murine leukemia viruses (MuLV) derived from mouse strains that develop spontaneous leukemia has led to the development of useful models for studying the critical events related to leukemogenesis.⁵³ Leukemogenesis by MuLV is a multistep process and the virus-induced changes during hematopoiesis are important in the initiation of disease. Several related viruses are grouped under MuLV, many of which were initially isolated from serial passage of murine tumors, including Gross murine leukemia virus (G-MuLV), Friend leukemia virus (FLV), Moloney murine leukemia virus (M-MuLV) and Rauscher murine leukemia virus (R-MuLV). While FLV and R-MuLV induce leukemia in erythroid and myeloid lineages, G-MuLV and M-MuLV cause T-cell leukemia in susceptible mouse strains.

Induction of erythroleukemia by Friend leukemia virus (FLV)

Since the discovery of acute erythroleukemia (acute form of myeloid leukemia caused by erythroblastic precursors) in mice infected by FLV⁵⁴ in the early 1950s, FLV-induced disease models have evolved as powerful tools to study the susceptibility of hematopoietic progenitor cells to retroviral infections.^{55,56} FLV is known to have two distinct viral components: A replication-competent Friend murine leukemia virus (F-MuLV) and a

replication defective component known as the Friend spleen focus forming virus (F-SFFV). The replication-competent F-MuLV is the non-pathogenic component of the viral complex and serves as a helper virus to SFFV, which is pathogenic by itself and responsible for the induction of acute erythroleukemia in mice.^{57–59} Infection of susceptible mouse strains with FLV leads to the development of acute erythroleukemia which is characterized by a rapid proliferation of pre-erythroblastic cells in the spleen that can be detected as early as 30 hours following viral infection, which is followed by the appearance of distinct foci of proliferating cells on the spleen, 10–12 days post-infection.^{54,60,61} These events represent the early hyperplastic (proliferative) phase of the disease and are a direct result of FLV infection of erythroid precursor cells. Two to three weeks post-infection, a clonally transformed population of pre-erythroblastic cells emerges in the spleen, causing hepatosplenomegaly and metastasis into peripheral organs such as the liver.^{62,63} The infected animal ultimately dies due to splenic rupture. These events represent the late oncogenic phase of the infection, which occurs as a consequence of FLV-mediated transformation of a small subpopulation of erythroid progenitor cells.

SFFV represents the pathogenic component of FLV and can infect a variety of hematopoietic cells, though early erythroid lineage-specific precursors are the primary targets of infection.^{60,64} The effect of SFFV can be divided into two distinct phases, correlating with the two phases of disease development: The early phase characterized by rapid proliferation of erythroid precursors (erythroid hyperplasia), and the late phase characterized by a clonal outgrowth of a subpopulation of the erythroid precursors. Erythroid hyperplasia is mediated by SFFV-induced alterations of the normal growth and differentiation profile of erythroid progenitor cells. The proliferation and differentiation of erythroid cells is tightly controlled by the erythropoietin (Epo) signaling pathway, which is initiated via binding of Epo with its receptor, Epo-R.⁶⁵ Erythroid hyperplasia is induced by SFFV in the absence of Epo.^{60,66,67} The envelope gene of SFFV, which encodes for a 55 kDa glycoprotein (gp55),^{68,69} has been shown to be crucial for the induction of erythroid hyperplasia during the early phase of SFFV infection in murine models.⁷⁰ Following SFFV infection of erythroid precursors, gp55 binds to the erythropoietin receptor, Epo-R,⁷¹ which is ubiquitously expressed in all erythroid progenitors, including the

main targets for SFFV, the late BFU-E and CFU-E cells.^{60,64} The binding of Epo-R by gp55, mediated by their respective transmembrane domains, results in Epo-R activation and promotes Epo-independent proliferation, differentiation and expansion of the erythroid progenitor cells.⁷²⁻⁷⁴ Several signaling pathways and molecules are activated downstream of the Epo-R and many of these, such as the JAK/STAT, Ras/Raf/mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3-kinase (PI3K)/Akt pathways, are constitutively activated in Epo-R-expressing cells infected with SFFV.^{65,75-77} It was initially thought that the constitutive activation of the Epo-R signaling pathway by gp55 was primarily responsible for SFFV-induced hyperplasia. However, gp55 has also been shown to interact and constitutively activate a truncated form of the stem-cell receptor kinase (sf-Stk).^{78,79} Although the exact role of sf-Stk in normal erythropoiesis is unknown, studies indicate that sf-Stk activation, through its interaction with gp55, appears to contribute to the development of erythroleukemia in mice.⁸⁰ The importance of the sf-Stk downstream signaling pathway in the initiation and maintenance of SFFV-mediated transformation has been established in SFFV-infected fibroblasts, outlining the importance of this pathway in SFFV leukemogenesis.^{81,82} Thus, both activation of Epo-R and sf-Stk pathway by binding of gp55 leads to a block in the normal differentiation pattern of erythroid progenitor cells, resulting in hyperproliferation and subsequent transformation in infected animals.

Subsequent to the formation of erythroid hyperplasia, the later leukemic phase of SFFV infection is manifested by clonal proliferation of a small subpopulation of the malignant erythroid precursor cells. These clonal subpopulations of erythroid precursor cells are characterized by the integration of SFFV into a specific cellular gene, *PUI.1*, resulting in the alteration of its transcriptional activities.^{83,84} Although the initial interactions of SFFV gp55^{env} with the Epo-R and sf-Stk signaling pathways are important for the early deregulation and proliferation of erythroid progenitors, these events are not sufficient for inducing malignant transformation of erythroid precursor cells. SFFV-mediated transformation of erythroid precursors is achieved through insertional mutagenesis, a method usually employed by non-acute transforming retroviruses. Analysis of malignant cells from SFFV-infected mice indicates that the

provirus predominantly integrates into a specific region of murine DNA known as *sp1* (SFFV-proviral-integration-site-1).^{84,85} Integration leads to the re-arrangement and transcriptional activation of the *sp1* gene by the SFFV-LTR, resulting in *sp1* overexpression.^{86,87} The *sp1* gene encodes for the transcription factor *PU.1*, which is a member of the *ets* family of transcription factors expressed in all hematopoietic cells with particularly high expression levels in B cells and macrophages. *PU.1* expression has been shown to play an important role in the regulation of hematopoiesis.^{88,89} Dysregulation of *PU.1* expression had been linked to the development of hematopoietic malignancies, including the transformation of myeloid cells.⁹⁰ During hematopoiesis, *PU.1* is required for hematopoietic development along both the lymphoid and myeloid lineages but is down-regulated during erythropoiesis. Studies done in knockout mice have shown that perturbation of *PU.1* expression results not only in the loss of B lymphoid and macrophage development but also delayed T lymphopoiesis.^{91,92} Additionally, *PU.1* also supports self-renewal of hematopoietic stem cells by regulating the multilineage commitment of multipotent hematopoietic progenitor cells, thereby maintaining a pool of pluripotent HSCs within the bone marrow.^{93,94} Importantly, *PU.1*, which is expressed in low levels in erythroid progenitor cells, needs to be down-regulated for terminal erythroid development.^{95,96} Thus, retroviral activation of *sp1/PU.1* transcription results in the blockage of erythroid development and outgrowth of a clonal population of erythroid precursor cells.

The induction of multistage erythroleukemia by FLV is a two-stage process: A pre-leukemic stage known as “erythroid hyperplasia” and a leukemic phase referred to as “erythroid cell transformation.” The pre-leukemic stage is characterized by the infection and random integration of the SFFV virus into erythroid precursor cells, followed by the expression and subsequent surface transportation of the viral envelope glycoprotein gp55 to the surface of the infected precursors. Gp55 activates both the Epo-R and the sf-Stk signaling pathways, leading to a constitutive active signal for proliferation of undifferentiated erythroid progenitor cells independent of erythropoietin (Epo). Within the proliferating erythroid progenitor cell population are infected cells where the virus randomly integrates into the *sp-1* locus, leading the activation and overexpression of *PU.1*. The overexpression of *PU.1* in erythroid precursor cells leads to a

block in erythroid differentiation, and in conjunction with an inactivation of p53, leads to clonal expansion of these leukemic cells in susceptible mice.

Induction of T-Lymphoma by Moloney Murine Leukemia Virus

Moloney murine leukemia virus (M-MuLV) is a non-acute retrovirus that typically induces T-cell lymphoma after a latency period of 3–6 months.⁵³ Typically, the tumor cells have the phenotype of immature T cells (CD4⁻/CD8⁻ or CD4⁺/CD8⁺) although some tumors show a more mature surface phenotype (CD4⁺/CD8⁻ or CD4⁻/CD8⁺).^{97,98} This has led to the hypothesis that the virus might originally infect a hematopoietic progenitor or an immature T-cell that continues to differentiate post-infection, initially in the bone marrow and then in the thymus.^{53,99} Because T lymphocytes develop in the thymus from bone marrow-derived immature precursors (pro-thymocytes), it has been proposed by several investigators that a bone-marrow-thymus axis plays a very important role in the development of T-cell lymphoma induced by M-MuLV.^{97,100–102} One of the characteristic features of M-MuLV leukemogenesis is the formation of mink cell focus-inducing recombinant viruses (MCF), which arise by the recombination between the envelope gene of an infecting M-MuLV and endogenous MuLV proviruses present in susceptible mice strains.^{103,104} Several studies have indicated that MCF recombination is important for M-MuLV leukemogenesis and efficient disease induction was correlated with efficient early infection of the bone marrow and the appearance of MCF recombinants.^{105,106} This association also suggested that MCF recombinant formation and initial propagation may take place in the bone marrow of the infected mice,¹⁰⁷ and that establishment of pre-leukemic changes including defects in bone marrow hematopoiesis, thymic atrophy, and hematopoietic hyperplasia in the spleen, might be induced by MCF recombinants.^{108–110} During the preleukemic phase of the disease, preleukemic cells can be detected in the bone marrow and spleen but not in the thymus. This is highly suggestive of the fact that the first preleukemic events may arise in the bone marrow and that the initial target cells for M-MuLV infection might be bone marrow-resident progenitor cells such as pro-thymocytes rather than mature thymocytes. A subsequent study also found increased levels of immature splenocytes

during the preleukemic phase of M-MuLV infection in mice.¹¹¹ Collectively, this suggests that murine hematopoietic progenitors and other bone marrow-resident cells are infected during the early preleukemic phase of infection and the infected cells subsequently migrate into the spleen and thymus, a hypothesis supported by data from M-MuLV murine models such as Akr mice.¹⁰¹ It was also shown that injection of bone marrow cells from AKr mice leads to a more rapid development of leukemia in irradiated secondary mice, confirming that the virus first infects bone marrow resident cells rather than infection of mature thymocytes or splenocytes.¹⁰¹

Studies involving Gross murine leukemia virus (G-MuLV) which induces lymphatic leukemia after a long latency have also shown that the bone marrow-thymus axis is crucial for the development of leukemogenesis.³⁴ G-MuLV-induced leukemia cells are predominantly immature, lymphoblastic cells (CD4⁺/CD8⁺ or CD4⁻/CD8⁻). Removal of the thymus prior to injection of virus prevents disease development, thus suggesting a role of the thymic micro-environment in development and manifestation of leukemia.¹¹² In early studies involving G-MuLV, the removal of the thymus a month post-infection resulted in the development of a myeloid leukemia instead of a T-cell lymphoma, implying that bone marrow resident progenitor cells are initial targets for infection.^{33,34} Subsequent studies have shown that the end targets for G-MuLV infection may be the immature thymocytes that house the outer thymic cortex.¹¹³ In contrast to other retroviruses such as AEV or FIV, where the target cells associated with infection have been conclusively determined as pro-erythroid precursors, the early target cells associated with both G-MuLV and M-MuLV infection have not been definitively identified. Studies in mice indicate that hematopoietic progenitor and/or bone marrow-resident stromal cells may, indeed, be the first targets for infection.¹⁰⁶ When bone marrow cells from BALB.C mice were infected with a particular strain of M-MuLV and subsequently analyzed using hematopoietic colony forming assays, it was found that the hematopoietic progenitor cells were the most likely target for infection.¹¹⁴ However, other reports have suggested that osteoclasts or osteoclast progenitors in the bone marrow are the primary targets for M-MuLV infection.¹¹⁵ Nonetheless, these studies detected a subsequent spread of infection to hematopoietic progenitors, indicating that the infection of

hematopoietic progenitors in the bone marrow may result from a secondary spread of the virus from osteoclasts or other directly infected cells.

Although the identity of the initial target cell in the bone marrow for M-MuLV infection is still unknown, a two-stage leukemogenesis model for the development of M-MuLV-induced leukemia has been proposed.⁵³ In this model, the virus infects an animal twice, in the preleukemic (early) phase as well as the leukemic (late) phase of infection. During the early phase of infection, recombination and formation of MCF occur in the bone marrow, which results in the generation of virus that progressively infects various bone marrow-resident progenitor cells, including hematopoietic progenitors, stromal cells and osteoclast progenitors resulting in defects in the bone marrow stromal microenvironment as well as dysregulation of normal hematopoiesis. This is followed by a distinctive hyperplasia of the spleen at 4 to 8 weeks after infection.¹⁰⁸ The early infection of the bone marrow is thought to be essential for the establishment of the preleukemic state because hyperplasia of the spleen is thought to occur as a result of perturbation of normal hematopoiesis in the bone marrow. The splenic hyperplasia is the result of a compensatory extramedullary hematopoiesis because of diminished normal hematopoiesis in the bone marrow and plays an integral role in the establishment of the malignant process.^{105,110,116}

It recently has been shown that M-MuLV induces a significant reduction in B-lymphoid differentiation in the bone marrow of infected mice by reducing the viability of differentiating B lymphoid progenitor cells.¹¹⁷ This suppression was found to be selective because the myeloid differentiation potential of the hematopoietic progenitors was found to be enhanced. This correlates with previously published reports on the disruption of normal bone marrow hematopoiesis and thymopoiesis in M-MuLV-related SL-3 murine leukemia virus infected mice, leading to a significant increase in myeloid but not lymphoid progenitor cells.¹¹⁸ This reduction in viability of differentiated lymphoid cells may confer a selective advantage to undifferentiated lymphoid progenitors in the bone marrow of MuLV-infected animals, contributing to the establishment of a preleukemic state.

On the whole, this supports a model of M-MuLV leukemogenesis in which the virus actively replicates in dividing hematopoietic progenitors

as well as other bone marrow-resident cells. Bone marrow hematopoiesis is thereby altered, particularly with the generation and maturation of increasing numbers of hematopoietic progenitors and increasing numbers of both myeloid and lymphoid progenitor cells that exit the bone marrow and migrate to the spleen. Eventually the pro-thymocytic population migrates into the thymus, where the environment is more suitable for their continued expansion. This triggers the leukemic phase of the infection, whereby these pro-thymocytes are re-exposed to the virus during thymic differentiation. Secondary infection of these progenitors is followed by M-MuLV LTR-mediated activation of proto-oncogenes, including *c-myc*, *bmi-1*, *gfi-1*, *tpl-1* and *tpl-2*.^{119–123} Apart from proto-oncogene activation, other important events occur during the later phase of infection, such as autocrine stimulation of the IL-2 receptor, creating IL-2-independent T cells,¹¹⁰ and chromosomal trisomy in chromosome 15, which perturbs the *c-myc* proto-oncogene.¹²⁴

Studies of M-MuLV-induced leukemia have proven very useful in understanding multistep processes associated with viral leukemogenesis and also identification of key physiological and virological events associated with tumor progression. These include, most notably, the elucidation of the activation of proto-oncogenes in tumorigenesis and the critical insight into the role of infection of bone marrow-resident hematopoietic progenitor and stromal cells in the establishment of the preleukemic state. Infection of these cells leads to dysregulation of normal hematopoiesis, and subsequent proliferation of malignant hematopoietic progenitors. Interestingly, defects in hematopoiesis in humans frequently result in the establishment of a leukemic state.¹²⁵ Murine retroviral models that induce leukemic states, particularly FLV and M-MuLV, have emerged as powerful models to study leukemogenesis as evident in recent studies done in AML.^{126–132} These murine models can be successfully used to study the complex processes of signaling and transcriptional activation of key hematopoietic regulatory genes that ultimately lead to malignancies.

Human Immunodeficiency Virus Type-1

Human immunodeficiency virus type-1 (HIV-1) infection is usually associated with depletion of CD4⁺ T cells. However, patients with HIV-1 often

manifest a wide range of hematopoietic abnormalities, such as cytopenia, leucopenia, anemia, neutropenia, and dysplasia.^{133–138} One intriguing question is whether the presence and replication of HIV-1 in the bone marrow of infected patients directly or indirectly impacts the normal proliferation and differentiation of hematopoietic progenitor cells. For more than two decades, researchers have attempted to establish if HIV-1 is capable of directly infecting hematopoietic stem or progenitor cells. It has now been convincingly demonstrated that hematopoietic progenitor cells largely remain uninfected by HIV-1 *in vivo*.^{139–142} Moreover, even if a subset of hematopoietic progenitor cells is susceptible to HIV-1 infection, the effective suppression of multilineage hematopoiesis does not require latent or active infection; instead it is primarily mediated by cytokine dysregulation induced by the interaction of HIV-1 gene products with hematopoietic progenitor cells or bone marrow stromal cells.^{143–153} Nonetheless, the presence and replication of HIV-1 within the bone marrow of infected individuals not only may result in bone marrow failure and subsequent progression to AIDS, but can also lead to the generation of progeny cells that are more susceptible to infection as it facilitates the rapid spread of the infection.^{154–156}

HIV infection of hematopoietic progenitor cells: In vivo and in vitro

Regardless of their source and differentiation state, a significant population of bone marrow-resident CD34⁺ hematopoietic progenitor cells express both the receptor and coreceptors for HIV-1, CD4 and CXCR4, although the expression of the other HIV coreceptor, CCR-5 is variable.^{157–161} Since the first reported study of CD34⁺ HPC infection with HIV-1, there have been conflicting reports in the literature about the susceptibility of these cells to HIV-1 infection, both *in vitro* and *in vivo*.¹⁶² Analysis of hematopoietic progenitors in the bone marrow of HIV-1 seropositive patients at various stages of infection has shown that these cells remain substantially uninfected, suggesting that suppression of hematopoiesis in HIV-1-infected individuals is probably related to other factors.^{139–141} Furthermore, in rare cases where there was detectable infection of hematopoietic progenitors, HIV-1 infection was at levels

insufficient to explain its direct role in the development of AIDS-associated hematological malignancies.^{142,163,164} In the initial reports of detection of HIV-1-infected hematopoietic progenitor cells within the bone marrow cells of HIV-1-infected patients, there were concerns regarding the contamination of the CD34⁺ cell preparations with either HIV-1 infected T-lymphocytes or macrophages leading to false positive results.^{165,166} Use of animal models that resemble human HIV infection, such as the SCID-hu mouse (Thy/Liv model) or simian immunodeficiency virus infection in macaques, have shown that HIV-1 infection disrupts both lineage-restricted and multilineage hematopoiesis *in vivo*, as indicated by the rapid and severe decrease of human progenitor cells capable of differentiation into both erythroid and myeloid lineages.¹⁶⁷⁻¹⁷² These studies also suggested that depletion of early hematopoietic progenitor cells occurs in the absence of direct viral infection, indicating that the suppression of hematopoiesis during HIV-1 infection may result from indirect effects of viral replication in hematopoietic progenitor cells.^{140,164,167,173-182} It is possible that infection of CD34⁺ HPCs is a rare event *in vivo*, and even when it does occur, it might only infect a more mature subset of HPCs in patients who are in a more advanced stage of the disease. It can be inferred that direct infection of hematopoietic progenitor cells is not sufficient to explain the suppression of hematopoiesis observed in HIV-1-infected patients. Other factors, such as abnormal stromal microenvironment, including infection of stromal cells and alteration in the cytokine milieu resulting from these infection mediated events, may have an important role to play in the suppression of hematopoiesis.

It has been established that HIV-1 can infect bone marrow stromal cells and that subsequently these infected cells fail to provide the optimal milieu of cytokines and adhesion molecules required for hematopoiesis.¹⁵ HIV-1-infected bone marrow cells secrete large amounts of proinflammatory cytokines and chemokines, including TNF- α , IL-1 α , IL-6, MIP-1 and RANTES, all of which can have deleterious effects on hematopoiesis.^{143,145,146} TNF- α , in particular, is a potent negative regulator of hematopoiesis and can induce expression of functional Fas on CD34⁺ cells, resulting in apoptosis and a compromised ability to reconstitute both short- and long-term multilineage hematopoiesis.¹⁸³⁻¹⁸⁶ Thus, the reduced population of hematopoietic progenitors observed in the bone marrow of HIV-1-infected patients

might be associated with hematopoietic suppression resulting from viral infection of accessory cells, leading to impaired stromal function and alteration of the hematopoietic cytokine network.¹⁸⁰ Since some of these effects, particularly the restoring to normal levels of TNF- α and Fas expression, are reversible with antiretroviral therapy, it can be inferred that HIV-1 infection of bone marrow stromal cells plays an important role in hematopoietic suppression as these cells serve to maintain the hematopoietic cytokine network. A recent study has suggested that very low levels of infection in a cell that is highly pluripotent and proliferative such as hematopoietic progenitors, can serve as an important reservoir of the virus, and may become a major obstacle in the complete eradication of the virus, which has a tendency to re-emerge.^{187,188} In a recent review by Alexaki *et al.*, the importance of HIV infection of hematopoietic progenitor cells is highlighted and implicated in viral trafficking and dissemination, resulting in the development of HIV-1-mediated dementia, among other pathologies.¹⁵⁵ The authors also speculate that HIV-1-mediated dysregulation of hematopoiesis might result in the outgrowth of a monocytic subpopulation that is more highly susceptible to infection and can potentially traffic the virus into various organs and the CNS.

Similar to *in vivo* infection of hematopoietic progenitor cells with HIV-1, the infection of hematopoietic progenitor cells with HIV-1 *in vitro* has also been controversial. Hematopoietic progenitor cells, including stem cells, express both the receptor and coreceptor for HIV-1 on their surface^{159–161,189,190} and some studies have reported successful *in vitro* infection of CD34⁺ HPCs with HIV-1.^{162,191} Other groups, however, have failed to infect hematopoietic progenitors with either primary or laboratory-derived strains of HIV-1.^{163,178,192} A number of possibilities could account for these differences, including the purity of the CD34⁺ HPCs cell population as well as the time of exposure and the particular virus isolate employed. The purity of the primary hematopoietic progenitor cells is an important consideration. In early studies, CD34⁺ HPCs were a heterogeneous population of cells also containing more mature differentiated lineage-specific progenitor cells which are more susceptible to HIV-1 infection.^{175,190,191} Studies done using clonogenic colony forming assays of HIV-1-infected CD34⁺ hematopoietic progenitor cells have shown that the more mature hematopoietic progenitors (including CD34⁺/CD38⁺ cells)

are more susceptible to HIV-1 infection, indicating that primitive hematopoietic stem cells might be resistant to HIV-1 infection.¹⁹³ Later studies demonstrated that a small subpopulation of CD34⁺/CD38⁻ hematopoietic stem cells may retain susceptibility to HIV-1 infection, but true hematopoietic stem cells that are mitotically inactive are resistant to infection even though they express both the receptor and coreceptor for viral entry.¹⁹⁴ Collectively, these data indicate that CD34⁺ human hematopoietic progenitors are not susceptible to HIV-1 infection either *in vitro* or *in vivo*, and suggest that defects in hematopoiesis observed as a result of HIV-1 infection are related to an alteration of bone marrow and peripheral blood microenvironments by soluble HIV-1-specific gene products.

HIV-1-mediated hematosuppression: Role of viral protein gp120^{env}, Gag and Vpr

Most of the *in vitro* and *in vivo* studies involving HIV-1 and hematopoietic progenitor cells point to the fact that cytopenia and other hematological malignancies exhibited in HIV-1-infected patients is multifactorial and is primarily due to indirect effects of HIV-1 infection on progenitor cells. Studies done *in vitro* with HIV-1-infected CD34⁺ HPCs and in SCID-hu mouse Thy/Liv models have shown that HIV-1 can affect both myelopoiesis and erythropoiesis from HPCs without productive or latent infection.^{171,172} In addition, human bone marrow cells exposed to HIV-1 *in vitro* show suppression of erythroid burst-forming units (BFU-E) and granulocyte-macrophage (CFU-GM) colony formation, although the defects in colony formation were not attributed to a productive HIV-1 infection of CD34⁺ cells. These data indicate that HIV-1 can impair bone-marrow hematopoiesis, acting in part at the level of hematopoietic progenitor cells but without the need of a productive or latent infection.^{140,142,170,171,175,178} Heat-inactivated virus can induce similar effects on hematopoietic progenitor cell activity,^{150,151} confirming the observation that suppression of hematopoiesis does not require productive or latent infection. The ability of antibodies against HIV-1 surface envelope glycoprotein gp120 to negate some of harmful effects of HIV-1 infection on hematopoietic progenitor cells has highlighted the potential role of this

viral glycoprotein in hematopoietic dysregulation.^{148–151} Exposure of hematopoietic progenitor cells to gp120 leads to the upregulation of TNF- α in these cells, which induces cells to undergo apoptosis through activation of the Fas pathway. Upregulation of TNF- α expression can potently inhibit the growth and proliferation of hematopoietic progenitor cells,^{195,196} and may result in a loss of hematopoietic progenitor cells, as reported in HIV-1 seropositive patients.¹⁸⁰ Other HIV-1 proteins, such as Gag, have been shown to suppress colony formation from hematopoietic progenitor cells, while intracellular Vpr expression in CD34⁺ HPCs induces G₂/M cell-cycle arrest.^{29,30,152} Another HIV-1 protein Tat, which is actively secreted by infected cells and can be taken up by a broad range of uninfected cells, including hematopoietic progenitor cells, has been shown to induce secretion of various cytokines important for regulation of hematopoiesis, including TNF- α and TGF- β .^{197–201} Tat has been speculated to cause hematosuppression, most likely in conjunction with gp120, as they induce the secretion of a similar pattern of cytokines from affected cells. Nevertheless, since studies have failed to establish whether inductions of these cytokines occur as a result of infection or in response to conventional antiretroviral therapy, the role of these pro-inflammatory cytokines on the development of AIDS-related cytopenias has yet to be conclusively established.

A clear paradigm has only now begun to emerge on the impact of HIV-1 infection on hematopoietic progenitor cells arising from the research conducted over the last two decades. The ambiguous evidence for the existence of both HIV-1-infected progenitor cells in *in vitro* cultures and from HIV-1-infected patients could suggest that *in vivo* infection of progenitor cells occurs rarely, if ever. However, it is clear that viral infection of auxiliary cells such as stromal cells and differentiated lineage-specific hematopoietic cells as well as exposure to viral gene products can indirectly influence survival and growth of hematopoietic progenitors by adversely affecting the microenvironment within the bone marrow.^{135,181} The greatest impact of HIV infection on growth and differentiation of hematopoietic progenitor cells results from the capacity of the virus to infect and perturb the hematopoietic regulatory function of supportive cells such as bone marrow stromal cells, and not from its capacity to infect progenitors and stem cells themselves. Further studies in this area are

warranted to understand the role of these factors in the manifestation of HIV-1-mediated hematopoietic malignancies.

Human T cell Leukemia Virus Type-1 and the Development of Adult T Cell Leukemia/Lymphoma (ATLL): Potential Role of Infectious Leukemic Stem Cells

Human T-cell leukemia/lymphoma virus type-1 (HTLV-1) is the causative agent of adult T-cell leukemia (ATL), an aggressive CD4⁺ leukemia.³⁶ ATL is a rare T-cell malignancy characterized by hypercalcemia, hepatomegaly, splenomegaly, lymphadenopathy, the presence of proliferating CD4⁺-CD25⁺ the leukemia cells and infiltration of lymphocytes into the skin and liver. HTLV-1 causes ATL in a small percentage of infected individuals after a prolonged latency period of up to 20–40 years.²⁰² ATL is characterized by a monoclonal expansion of malignant CD4⁺CD25⁺ T cells which evolve from a polyclonal subpopulation of HTLV-1 infected CD4⁺ T cells. Although HTLV-1 can replicate through reverse transcription during the initial phase of infection, the viral genome is effectively replicated during proliferation of the infected cells.²⁰³ Typically HTLV-1 infected cells can persist for decades in patients and the infected cell population transitions from a polyclonal phase into a monoclonal expansion. There are four ATL subtypes: acute, lymphomatous, chronic, and smoldering. The first two subtypes are associated with a rapidly progressing clinical course with a mean survival time of 5–6 months. Smoldering and chronic ATL have a more indolent course and may represent transitional states towards acute ATL. The clinical features of ATL include leukemic cells with multi-lobulated nuclei called “flower cells” which infiltrate various tissues, including the skin and the liver with abnormally high blood calcium levels and concurrent opportunistic infections.

Although considerable progress has been made in understanding ATL biology, the exact sequence of events occurring during initial stages of malignancy, including cell types infected with HTLV-1, remains unclear. The primary target cells for HTLV-1 infection may not only influence HTLV pathogenesis, but sequestration of these cell types may allow the virus to effectively evade the primary immune response against infection. It has been previously reported by authors’ laboratory and other investigators that HTLV-1 can infect hematopoietic progenitor cells.^{204,205} It was hypothesized

that HTLV-1 can specifically induce a latent infection in CD34⁺ HPCs and can initiate preleukemic events in these progenitor cells.²⁰⁶ These cells could potentially provide a durable reservoir for latent virus in infected individuals. HTLV-1 infection of CD34⁺ HPCs may also induce perturbation of normal hematopoiesis, ultimately resulting in the outgrowth of malignant clones and development of ATL. It has been hypothesized that HTLV-1 infection of CD34⁺ HPCs may result in the generation of an “infectious leukemic stem cell.” Data from the authors laboratory indicate that HTLV-1 infection of CD34⁺ HPCs induces G₀/G₁ cell-cycle arrest and causes perturbation of hematopoiesis *in vitro*, an effect also mediated by the transduction of HTLV-1 oncoprotein Tax1.^{29,30,207} This is in stark contrast to the growth stimulation and transformation following HTLV-1 infection of mature CD4⁺ T cells. Infection of hematopoietic progenitor cells by HTLV-1 may establish important preleukemic events in HPCs, which ultimately manifests in HTLV-1 pathogenesis. *Ex vivo* infection of CD34⁺ HPCs and reconstitution of hematopoiesis in humanized SCID mice results in recapitulation of ATL, implicating the role of infected HPCs in the manifestation of HTLV-1 mediated leukemia.²⁰⁸

Tax1: The role of the HTLV-1 oncogene in ATL development

The molecular events that cause HTLV-1 infection to progress from clinical latency to T-cell malignancy are not fully understood, but it is generally believed that they involve the critical viral transactivator protein Tax1. HTLV-1 carries no cellular proto-oncogenes and the oncogenic potential of the virus is linked to Tax1, which is a 40 kDa protein that functions as a trans-activator of viral gene expression and as a key component of HTLV-1-mediated transformation.²⁰⁹ Apart from regulating viral gene expression through the 5′-long terminal repeat (LTR), Tax1 can induce or repress the expression of a large variety of cellular genes, including those encoding for cytokines, apoptosis inhibitors, cell cycle regulators, transcription factors and intracellular signaling molecules.^{210–212} Tax1 usually induces cellular gene expression through activation of transcription factors such as NF- κ B and cyclic AMP response element-binding protein/activating transcription factor (CREB/ATF).²¹³ Tax1 has also been shown to *trans*-repress transcription of certain cellular genes, including *bax*,²¹⁴ human β -polymerase,²¹¹ cyclin A,²¹⁵ *lck*,²¹⁶

MyoD,²¹⁷ INK4,²¹⁸ and p53.²¹⁹ Tax1 can induce immortalization and transformation of T cells *in vitro*^{220,221} as well as induce formation of a variety of malignancies in transgenic mice.^{222,223} It is noteworthy, however, that none of the Tax-transgenic mouse models showed mature CD4 T cell leukemia. Although Tax1 has been implicated in having a pivotal role in ATL development, precisely how Tax1 influences ATL development is not completely understood. One of the emerging views of HTLV-1 leukemogenesis and ATL development correlates with neonatal transmission and that HTLV-1 targets HPCs and immature human thymocytes which ultimately results in perturbation of normal hematopoiesis.^{29,204,205} Infection of HPCs results in skewing of hematopoiesis towards distinct cellular lineages and outgrowth of malignant clones with an eventual manifestation of ATL. HPCs may serve as a crucial target for HTLV-1 latency and an *in vivo* reservoir of infected cells, thus playing a pivotal role in the development of ATL.^{204,206} This hypothesis is supported by the development of lymphoma in Tax1 transgenic mice in which Tax1 expression was restricted to immature thymocytes through the use of the lymphocyte-specific protein tyrosine kinase (LCK) promoter.²²³ More importantly, this data indicates that Tax1 expression in early HPCs has a unique distinguishing role, which contributes to the induction of lymphoproliferative disease. The role of Tax1 in progenitor cells may include, but is not restricted to, cell cycle dysregulation and perturbation of hematopoiesis as have previously been reported (Fig. 2).^{29,207}

Cell cycle arrest in HPCs: Dysregulation of p21^{cip1/waf1} (p21), p27^{kip1} (p27) and survivin

Eukaryotic cell cycle progression in cells is regulated by sequential activation and inactivation of a series of cyclin-dependent kinases (CDKs) at different stages of the cell cycle. D-type cyclins (D1, D2 and D3) and cyclin E are involved in regulating G₁ progression and entry into the S phase. Tax1 plays a significant role in the dysregulation of cell cycle in HTLV-1-infected CD4⁺ T cells, especially in accelerating G₁ to the S phase transition by modulation of several cellular activities.²²⁴ Tax1 increases the levels of G₁ D cyclins and also activates transcription of CDK2 and CDK4,²²⁵ leading to the stabilization of cyclin D/CDK complexes.²²⁶ Tax1 has also been shown to bind to the hyperphosphorylated form of retinoblastoma protein (pRB), facilitating its

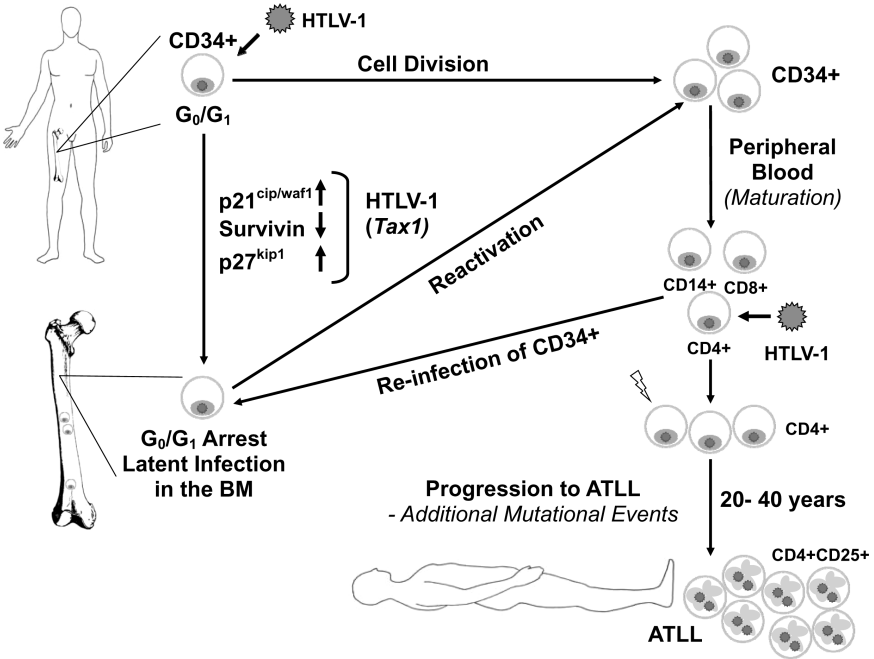


Fig. 2. Model of HTLV-1 latency within the bone marrow of infected patients.

proteosomal degradation.²²⁷ The phosphorylation and degradation of RB frees the E2F1 transcription factor, accelerating cell-cycle transition from G1 to S. Thus, the ability of Tax1 to generate an abundance of activated cyclin D/CDK complexes and to promote RB phosphorylation and subsequent E2F release, results in committed G1 egress in HTLV-1-infected CD4⁺ T cells.

Paradoxically, Tax1 expression also induces G₁/S cell cycle arrest, leading to a senescence-like state in both cultured mammalian cell lines and primary human CD34⁺ HPCs.^{207,228} Likewise, expression of Tax1 in *Saccharomyces cerevisiae* leads to growth arrest and loss of cell viability.^{229,230} Intriguingly, in addition to increasing the levels of cyclins and CDKs, Tax1 also increases the levels of CDK inhibitors p16^{Ink4}, p21^{cip1/waf1} and p27^{kip1} in infected cells.^{29,30,228,231} Overexpression of p21 inhibits two critical checkpoints in mammalian cell cycle, namely G₁/S and S/G₂, through p53-independent and dependent pathways.²³² Moreover, p21 and p27 are the key contributors in cell-cycle regulation of CD34⁺ HPCs.^{233–235}

Cell cycle and differentiation is highly regulated in CD34⁺ HPCs, with a majority of CD34⁺ HPCs residing in quiescence and demonstrating unique expression patterns of CDKs, cyclins, and CDK inhibitors. The CDK inhibitors p21^{cip1/waf1} (p21) and p27^{kip1} (p27), in particular, have been shown to be key contributors in restricting cell cycle entry from G₀ and maintaining quiescence in CD34⁺ HPCs.^{233–235} We have previously shown that during HTLV-1 infection, induction of G₀/G₁ cell cycle arrest and suppression of multilineage hematopoiesis in HPCs is attributed to the concomitant activation of p21^{cip1/waf1} and p27^{kip1} in these cells by Tax1.^{29,30,207} Although Tax1 usually induces cellular gene expression by activation of transcription factors such as NF- κ B and CREB/ATF, it has recently been suggested that Tax1 dysregulation of p21 and p27 might be mediated by mechanisms independent of NF- κ B activation.²³⁶ The reported absence of NF- κ B activity in CD34⁺/CD38⁻ hematopoietic stem cells²³⁷ allows speculation that the hematopoietic stem cells may provide a unique microenvironment for HTLV-1 infection, which stands in stark contrast to the cellular environment provided by mature CD4⁺ T lymphocytes. It may be inferred that Tax1-mediated cell cycle dysregulation is cell-type specific, inducing cell cycle arrest in HPCs while concurrently maintaining the ability to activate cell proliferation in CD4⁺ T cells.

Survivin, originally identified as a member of the inhibitor of apoptosis protein family, has recently been implicated in regulating hematopoiesis, cell cycle control and transformation.^{238–241} Survivin is expressed in normal adult bone marrow and in CD34⁺ HPCs and its expression is upregulated by hematopoietic growth factors.²⁴² Notably, survivin has been shown to be a key mediator of early cell cycle entry in CD34⁺ HPCs and regulates progenitor cell proliferation through p21-dependent and -independent pathways.²⁴³ These data implicate survivin as an integral cellular factor which regulates multiple aspects of hematopoiesis. The authors have recently demonstrated that HTLV-1 infection of CD34⁺ HPCs suppresses hematopoiesis as a result of induction of G₀/G₁ cell cycle arrest by modulation of not only p21 but survivin gene expression.³⁰ Notably, CD34⁺/CD38⁻ hematopoietic stem cells demonstrate sensitivity to cell-cycle arrest following HTLV-1 infection in comparison to more mature CD34⁺/CD38⁺ hematopoietic progenitor cells, suggesting that HTLV-1 may facilitate a latent infection in these cell types *in vivo* by arresting cell cycle and inducing quiescence.

CD34⁺ HPCs have previously been shown to be cellular targets for viral infection, including CMV, HHV-6, HHV-8, measles virus, and MuLV.^{12,18,20,25,26,117,187,244} Cells residing in the bone marrow, HPCs and HSCs, have previously been proposed as sites of retroviral latency.²⁰⁶ It has been demonstrated that CMV selectively establishes latency in CD34⁺/CD38⁻ stem cells, resulting in altered cellular gene expression patterns and inhibition of clonogenic colony formation activity (CFA) *in vitro*.²⁰ These results are precedents for modeling the role of HTLV-1-infected HPCs in the manifestation of T cell leukemia. It has been demonstrated that high levels of proviral HTLV-1 DNA exist in the bone marrow (BM) of HTLV-1 infected patients.²⁴⁵ Exposure of peripheral blood to HTLV-1 results in CD4⁺ T cell infection and this cell population routinely traffics to the BM as part of normal immune surveillance. Trafficking of infected CD4⁺ T cells into the BM during the acute phase of infection may result in HTLV-1 infection of the CD34⁺ hematopoietic progenitor cells, including the bone marrow resident HSCs. It can be speculated that human HPCs provide a unique microenvironment in contrast to the cellular environment provided by transformed cell lines for cell cycle arrest and suppression of hematopoiesis.

ATL is etiologically linked with neonatal or perinatal transmission of HTLV-1 infection and the disease develops decades after the initial infection. HTLV-1 mediated suppression of multilineage hematopoiesis and cell-specific cell cycle arrest in CD34⁺ HPCs has been reported and identifies a unique mechanism by which this human retrovirus establishes viral latency and avoids immune surveillance in humans, thus accounting for the relatively long persistence of HTLV-1 infection demonstrated in ATL patients. The sequestration and concomitant differentiation of HTLV-1-infected HPCs may be a mechanistic pathway which allows the virus to evade immune surveillance while concurrently providing a continuing supply of infected cells *in vivo*. This is also in accordance with previous reports that the bone marrow, a site enriched with CD34⁺ HPCs, is a target for HTLV-1 infection.^{206,245} Ongoing studies in our laboratory have outlined the relevance of HTLV-1 infection of CD34⁺ HPCs *in vivo*. HTLV-1 infection of CD34⁺ HPCs recapitulates ATL disease in humanized SCID mice, showing concurrent hyperproliferation infected HSCs in the bone marrow. This suggests that

HPCs and HSCs represent target cells for maintaining HTLV-1 infection for extended periods of time *in vivo*, leading to the development of lymphoproliferative disease and may result in the establishment of infectious leukemic stem cells.

CONCLUSIONS

Retroviruses can establish latent infection in HPCs and HSCs, resulting in perturbation of hematopoiesis and induction of viral pathogenesis as have been discussed here. Retroviral infection of HP/HSCs results in wide-ranging consequences, including suppression of hematopoiesis and establishment of viral latency in the relatively immune-privileged site of the bone marrow. Moreover, some retroviral infections specifically target more mature lineage-specific hematopoietic progenitors, including those of the erythroid or lymphoid lineages, leading to the development of hematological malignancies. Study of retroviral pathogenesis has helped scientists decipher some of the basic molecular events associated with hematopoiesis and differentiation. More research is warranted in the future to further our understanding of these mechanisms and to better understand how retroviruses infect, survive and cause disease in humans and other vertebrates.

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