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HUMAN IMMUNODEFICIENCY VIRUS AND THE HEMATOPOIETIC REPERTOIRE: IMPLICATIONS FOR GENE THERAPY

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1. ABSTRACT

The human immunodeficiency virus (HIV) infects primarily the hematopoietic and immune systems. At the onset of infection, an initial activation of the immune system occurs, with a subsequent suppression thereafter due to direct viral infection of cells, inhibitory effects of HIV proteins, an altered microenvironment with cytokine imbalance, and increased apoptosis of both infected and non-infected cells. The CD34+ hematopoietic stem cell, however, remains free of infection. Novel methods in gene therapy utilize viral vectors that can introduce genes with good efficiency into the non-dividing stem cell. Therefore, HIV-resistance genes can be introduced into stem cells using these vectors. This confers resistance to infection to their respective progeny, and concurrently allows for repopulation of the immuno-hematopoietic repertoire. Applications of this technology to the patient infected with HIV are discussed in the context of myeloablative therapy and stem cell rescue.

2. INTRODUCTION

The study of HIV has made a significant contribution to the increased understanding of human immune responses, and in particular the role of chemokines and their receptors in these responses (1-3). As most patients with HIV also exhibit other hematologic abnormalities, it has also lead to a greater appreciation for the bone marrow microenvironment, and the cytokines responsible for its homeostasis (4). Furthermore, gene therapy with lentiviral vectors, now a mainstay for the transfection of quiescent hematopoietic stem cells (HSC), is likely a consequence of the understanding of mechanisms of HIV infection and propagation (5).

This work attempts to unite these concepts and offer a picture of the disruption in the hematopoietic and immune homeostatic mechanisms which occur in HIV. Using these concepts, options for treatment using gene therapy for HIV are discussed.

3. HIV AND HEMATOPOIESIS, AN OVERVIEW

The hematopoietic system is the primary target of HIV infection, and as such, reflects the course of the disease. More severe cytopenias are seen in patients with more advanced disease (6, 7). Some degree of anemia, thrombocytopenia, lymphocytopenia, neutropenia, and/or monocytopenia is present in most patients with HIV infection. These effects can be either from chemotherapy for HIV itself and its related conditions, opportunistic and intercurrent infections, or from direct effects of the virus on the different components of the blood and bone marrow. Recently it has become evident that viral infection in and of itself has devastating consequences on hematopoietic and immunologic homeostatic mechanisms (4, 8).

In the blood, isolated thrombocytopenia is often the first sign of HIV-1 infection, with anemia and granulocytopenia occurring later in the disease (9, 10). Many infected patients with low platelet counts harbor normal or increased megakaryocytes in the bone marrow, and elevated levels of anti-platelet antibodies, typical of idiopathic thrombocytopenic purpura (ITP) (9). HIV infected patients also display an increased incidence of autoimmune disorders (2). The increase in polyclonal autoantibody production due to B-cell hyperstimulation is evident in bone marrow examinations by an increase in

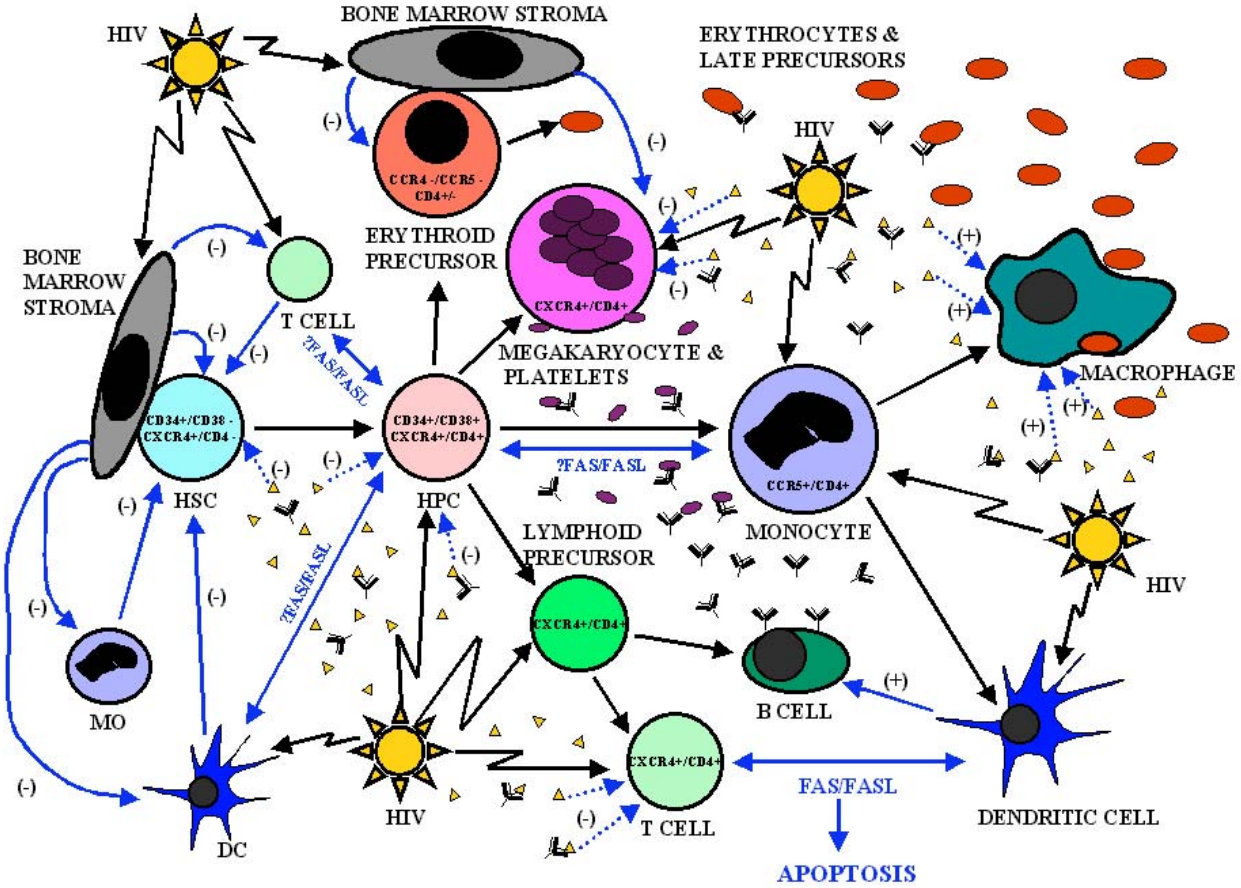

Figure 1: The hematopoietic microenvironment and hiv infection. Lineage commitment from the hematopoietic stem cell (HSC) and hematopoietic progenitor cell (HPC) is shown with the respective surface receptor expression relevant to states of HIV infection and hematopoietic ontogeny. Solid black arrows represent progressive lineage commitment, whereas lightening-bolt black arrows represent HIV viral entry into cells. Yellow triangles show HIV proteins, such as Vpr, and gp-120, which affect cell functions, and black Y-figures denote antibodies which are produced in increased amount due to B-cell hyperstimulation. The antibodies shown are for platelets and erythrocytes, leading to increased destruction and sequestration in HIV, and for gp-120, required for crosslinking to occur on the target cell for either inhibitory or stimulatory effects to occur as indicated by the (-) and (+) signs, as well as by the dotted blue arrows. Some of these viral proteins do not require crosslinking with antibodies, again denoted by the dotted blue arrows and the (-) and (+) signs. Inhibitory signals in the form of cytokines are represented by the solid blue arrows and the (-) sign. Fas/FasL signalling with resultant apoptosis is shown by the double solid blue arrows. See text for more details.

plasma cells and lymphoid aggregates (9). However, this immune stimulation is countered by immune inactivation due to direct HIV infection of CD4+ T cells and antigen presenting cells. The outcome is a shift in immune homeostasis from Th1-type responses, producing interferon (IFN)-γ, interleukin (IL)-2, IL-12, IL-15, and IL-18, to Th2-type which upregulate the cytokines IL-4, IL-5, IL-6, IL-10, and IL-13 (3). This immune dysregulation compounds the direct viral pathogenic mechanisms responsible for the ineffective hematopoiesis seen in HIV (2-4, 9, 10).

The mechanisms responsible for the hematopoietic abnormalities and dysfunction which occur in patients with HIV infection are multifactorial. Apart from the opportunistic infections, malignancies, vitamin and mineral deficiencies and myelosuppressive pharmacotherapy common in this population, HIV itself causes dysregulation of bone marrow function:

1. HIV infection of bone marrow stroma and supporting structures provides a hostile environment for the maintenance and differentiation of the hematopoietic stem cell.
2. Once infected, committed progeny undergo apoptosis, or programmed cell death, due to upregulation of the Fas/Fas Ligand (Fas/FasL) pathway.
3. Stimulation of autoantibody production targets and destroys normal platelets, erythrocytes, and neutrophils.
4. HIV proteins themselves, apart from infection, inappropriately inhibit and/or stimulate normal cell functions.

These issues are discussed below and in figure 1

in the context of the different components which constitute the bone marrow space, and with respect to their relevance in gene therapy.

4. HIV AND THE BONE MARROW MICROENVIRONMENT

4.1. CD34+ Stem Cells

Although much controversy still exists regarding the ontogeny of the HSC (11), the clinically relevant research in stem cells and, in particular stem cell transplant, assumes that the earliest HSC bears the CD34 marker, lacks CD38, and is in the out-of-cycle G_0 state (12-16). The hematopoietic progenitor cell (HPC) is a CD34+ cell able to produce colonies of committed cells of all lineages yet is unable to provide long-term repopulation (14,16).

Early reports indicated that HIV infected the HSC resulting in decreased colony growth (17,18). However, more recent data shows that the CD34+/CD38-, G_0 HSC remains free of infection, and that decreased colony growth is due to HIV infection of the microenvironment (4). The apparently conflicting evidence for infection of the HSC and HPC with HIV can be explained. The method of CD34+ cell isolation, the level of purity obtained, and the exclusion of possible contaminating cells that are CD34+ but are not necessarily HSC or HPC, such as endothelial cells, can explain much of the disparity (17-37). If HSC/HPCs purified from the bone marrow samples of HIV infected individuals are plated in low density and their respective colonies contain cells which express or contain viral elements at any stage, and none which do not harbor virus, only then can these cells be considered infected progenitors (4). However, even in studies which did not use such strict criteria to define infected progenitors, those that did report HIV infection of CD34+ cells did so only at a very low level, and not one that could explain the hematopoietic derangements that occur in these patients (4, 9, 17-37). Furthermore, the multilineage suppression that occurs with HIV infection can be reversed with combination antiretroviral therapy, showing that the HSC/HPC is preserved (27).

In attempts to infect the CD34+ HSC *ex vivo* from normal bone marrow, the same controversy exists. This disparity may be due to the difference in viral isolates used for infection, or due to the difference in the conditions of the culture (4, 9, 18, 28, 32, 38-43). In single cell culture studies with saturating amounts of growth factors, exposure to HIV results in a low degree of infected colonies (less than 25%) (44). More importantly, however, the capacity of the infected progenitor to undergo differentiation is not affected. Therefore, even if the HPC is able to be infected by HIV, either *in vitro* or in *vivo*, the degree of infection is not such that would limit the growth potential of the progenitor, and, given this data, is not likely to cause apoptosis directly.

When specific subsets of progenitor and precursor cells are evaluated for their infectability and capacity to harbor HIV infection, a clearer picture emerges. For better definition, cells are sorted based on their surface markers and expression of chemokine receptors as well as their capacity as long-term culture initiating cells (LTCIC). The CD34+/CD38- population of HSCs is not able to be infected with HIV-1 or HIV-2 *in vitro* and does not harbor virus in LTCIC assays (45). However, both strains of HIV do infect the more committed CD34+/CD38+ cells, correlating also with the upregulation of CD4, CCR5, and CXCR4 on their surface (45). Furthermore, functional separation of the CD34+/CD38- subset into G_0, non-cycling cells, again shows that these cells are free of HIV-1 infection both *in vivo* and *in vitro*, even though all subsets of CD34+ cells express CXCR4 at high frequencies (46, 47). A small subpopulation of these cells are also CD4+, and are still resistant to HIV infection at the level of viral entry. This suggests a third, undefined, coreceptor necessary for HIV infection absent from the HSC (46) or that receptor/coreceptor ratios on the surface of the HSC are inadequate for viral entry at this stage.

Although it is clear that the frequency of HPCs in the bone marrow and peripheral blood decreases as the disease progresses, this is not a consequence of direct viral infection (4, 9, 48). Rather, inhibitory effects of T-cells (34, 50) and other accessory cells (23, 37) through cytokine signaling or upregulated Fas/FasL apoptotic signals (3), inhibitory HIV membrane and regulatory proteins (29, 49), and a still ill-defined inherent defect in the HSC/HPC itself (9, 22, 37, 48) seem to be responsible. This inherent defect may be due to defective or absent cell surface proteins resulting from an altered bone marrow microenvironment.

The inhibitory effect of HIV on the hematopoietic environment in the absence of active infection plays an important role in the dysregulation of progenitor and stem cell homeostasis (37, 40, 41). The interaction between the viral envelope glycoprotein and the cell membrane (52), and the activity of the regulatory genes, *tat* and *nef* (29), is sufficient to produce defects in growth either directly or through the upregulation of inhibitory cytokines (9). Furthermore, the crosslinking of gp120 with antibodies against gp120 found in patient sera, enhances the growth inhibitory effects and apoptosis (52, 53).

Thus, in HIV disease, the evidence supports that the CD34+ HSC/HPC is not infected *in vivo* in early disease. Rather, the inhibitory effects at the progenitor cell level occur as a result of viral protein-membrane interaction, and secondary inhibition from infected accessory cells in the bone marrow microenvironment (figure 1).

4.1. Bone Marrow Stromal Elements

The bone marrow microenvironment is composed of various elements arranged in a lattice-like scaffolding upon and with which the complex interactions of hematopoiesis take place (54, 55). An important component of the microenvironment is the bone marrow stroma composed of fibroblasts, adipocytes, endothelial cells, macrophages, and extracellular matrix (55). Not only does the stroma act as a support structure for important interactions to occur, but it also provides humoral (cytokines) and cellular (adhesion proteins and receptors)

signaling vital to normal bone marrow growth, regulation, and function (54, 55).

In HIV infection, the bone marrow stroma could be affected directly with cytopathic consequences, or through altered cytokine profiles and responses leading to dysregulated hematopoiesis (9). Similar to the studies examining the HSC/HPC in HIV infection, controversy exists with regard to the infectability of bone marrow stroma with HIV and whether this impacts hematopoiesis (4, 9). This disparity lies in the HIV isolates used, the culture conditions set, and the type of cells studied in the culture (25, 41, 56-61). Those studies which utilize culturing techniques favoring a fibroblast monolayer generally do not show any deleterious effects of *in vitro* HIV infection on hematopoiesis, and indeed, do not show infection at all in *in vivo* studies of HIV infected patients (9, 58). However, those culture techniques which favor the overgrowth of a microvascular endothelial cell (MVEC) layer alone or in addition to fibroblasts, do show significant impact of HIV infection resulting in dysregulated hematopoiesis (4, 60, 61). Given the dynamic nature of the microenvironment, the latter of the two types of culture, although still very artificial, is more representative of the *in vivo* state.

In HIV-1 infected patients, bone marrow MVECs are found to be infected (60) even in early disease, whereas, fibroblasts are not (9). These MVEC enhanced stromal cultures constitutively produce the growth factors IL-4, IL-6, granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), tumor necrosis factor (TNF)-α, transforming growth factor (TGF)-β, and steel factor normally. However, when exposed to IL-1α, they are unable to produce appropriate amounts of G-CSF and IL-6 in response (60). Hence, HIV-1 infected stromal cell layers with only a very minor amount of MVECs and monocytes/macrophages (2% of each), cannot support hematopoiesis and infection results in a decline in clonogenic progenitor cells (61). However, stroma which is genetically modified with a decoy to HIV and therefore resistant to infection, does not show the same hematopoietic dysregulation (61). This indicates that even early in HIV disease, bone marrow MVECs are infected and can act as a reservoir for the virus. Furthermore, MVECs are unable to respond appropriately to humoral signaling within the microenvironment during times of stress, such as in response to inflammatory stimuli or to enable recovery from myelosuppression. Thus, inappropriate cytokine signaling and responses between infected and uninfected cells (that is, between MVECs and fibroblasts) may result in a microenvironment hostile to progenitor growth and differentiation (4).

Since the bone marrow stroma and microenvironment is a complex three-dimensional system, the full impact of HIV infection of the stroma on hematopoeisis cannot be understood until suitable *ex vivo* culture conditions can emulate bone marrow dynamics and structure (62, 63). However, the current available culture methods and data strongly suggest that HIV infection of elements of the bone marrow stroma results in an altered response pattern to signals important in progenitor cell maintenance, growth and differentiation, and can in part explain the decrease in progenitors seen in later disease.

4.3 Committed Hematopoietic Lineages

Even with the relative sparing of the HSC, infection of more mature CD34+/CD38+ progenitor cells with HIV can still result in infected progeny of all lineages (see section 4.1 above). Despite this "primary" route of infection, "secondary" infection can occur once cells have committed to their respective lineages, as long as the necessary HIV receptor (CD4) and chemokine coreceptors (mainly CCR5 and CXCR4) are present and function normally to allow for HIV entry (64). It is now well established that the coreceptor CXCR4 is required for entry of T-tropic strains of virus, or those HIV-1 strains which mainly infect T-cell lines, and CCR5 is required for the M-tropic strain of HIV-1, which infects primary monocytes and macrophages. There are, as well, dual-tropic strains which can utilize both coreceptors for viral entry to infect both macrophages and T-cell lines (64). Other chemokine receptors which mediate fusion of HIV-1 by functioning as coreceptors include CCR2b, CCR3, CCR8, STRL33, GPR1, CX_3CR1, ChemR23, APJ and GPR15 (47, 64).

Chemokine receptor analysis on committed hematopoietic progeny shows that, aside from the known presence of CCR5 on monocytes and macrophages and CXCR4 on T-cells, both CCR5 and CXCR4 coreceptors, in addition to CD4, are present on megakaryocytes and granulo-macrophage cells. However, only T-tropic viruses (using the CXCR4 coreceptor) can infect megakaryocytes and M-tropic viruses (using the CCR5 coreceptor) can infect granulo-macrophage cells (47). This "selective" tropism, despite both coreceptors being present, may result from the differential affinity for receptor/ligand and their interactions, the CD4-coreceptor density and ratio on the surface of the cells in question, as well as post-viral entry determinants (47, 65). Dendritic cells can be infected by both M-tropic and T-tropic HIV viruses *in vitro* as they express numerous chemokine receptors on their surface (66).

Regardless of the type of HIV strain able to infect megakaryocytes and granulo-macrophage cells, the fact that they are susceptible to infection is evidence for a primary cause of their respective cytopenias. In particular, the etiology of thrombocytopenia in HIV+ patients can now be attributed to primary HIV infection of the megakaryocytes in the bone marrow as well as inhibitory signals from an infected microenvironment, with resultant ineffective thrombopoiesis (67). This is compounded by anti-platelet antibodies and early destruction/decreased survival time in the periphery (10, 67) from a dysregulated immune system. Also, HIV-1 infection upregulates Fas and FasL in infected monocytes (68), macrophages and lymphocytes (8) and provides apoptotic signals to both infected and uninfected cells expressing Fas on their surface, such as monocytes, dendritic cells, lymphocytes, and possibly progenitor cells (4, 8). Whereas Th1 cells are more susceptible to apoptosis, Th2 cells are more susceptible to HIV infection (8). Thus, in general,

accelerated death of Th1 cells from an infected microenvironment due to Fas/FasL signaling shifts the immunologic paradigm to Th2, which in turn leads to total T-cell depletion due to primary HIV infection and T-progenitor cell depletion (69).

Cells of the erythroid lineage express predominantly CCR2, low levels of CD4, and no CXCR4 or CCR5 receptors, and are therefore unable to be infected by either M- or T- tropic strains of virus (47). This indicates that direct HIV infection of erythroid cells with these strains of virus likely does not occur and is not a major cause of HIV-related anemia. However, it remains to be seen whether more restricted virus strains impact in the pathogenesis of erythroid infection with HIV using the CCR2 coreceptor, although the low levels of CD4 on their surface is likely insufficient for viral membrane fusion (65). The etiology of HIV-related anemia is more likely a secondary effect from the dysregulated bone marrow microenvironment and immune system. Moreover, HIV-1 Vpr protein expression, without active HIV infection, induces erythrophagocytosis prematurely by cellular binding with mononuclear phagocytes (70). Thus, inhibitory signals from the infected stroma and other accessory cells (figure 1), as well as anti-erythrocyte antibodies from hyperactivated B-cells and HIV extracellular proteins, such as Vpr, act in synergy to suppress and destroy both erythroid precursors and mature cells (4, 10, 70).

4.4. Cytokines

The cytokine milieu at cellular interfaces determines the type, degree, and longevity of a particular immune response and provides growth and differentiation signals for hematopoiesis. An acute or chronic shift in this cytokine pattern, either from a distance from infected lymphoid tissues, or within the local microenvironment, can result in profound immuno-hematologic dysregulation as seen in HIV disease.

When leukocytes from HIV-infected individuals are stimulated *in vitro*, they exhibit a cytokine profile low in IL-2, Interferon (IFN)-γ, and IL-12, but increased in IL-4 and IL-10 (8), consistent with a Th1 to Th2 cytokine shift. Furthermore, increased production of GM-CSF, IL-3, IL-4, and IL-10 can result in bone marrow hyperplasia and lymphoid aggregates characteristic of the initial myeloid and B-lymphocyte stimulation seen in early HIV infection (9). Also, IFN-γ upregulates the expression of CXCR4 on a subpopulation of granulo-monocytic precursors (47), and CCR5 is upregulated by IL-10 (71) but down-regulated by GM-CSF (72) and IFN-β (73). Mononuclear cells infected with HIV-1 *in vitro* upregulate TNF-α (74), TGF-β1 (75), which both suppress hematopoiesis, and IL-6 (76) that can enhance colony growth and stimulate B-cells. Therefore, HIV infection of cells in the bone marrow microenvironment causes a disorganization of cytokine signals which, in addition to causing primary dysregulation, can also regulate chemokine receptor expression and thereby modulate HIV replication *in vivo*.

Aside from infection per se, HIV proteins gp120 and Tat affect hematopoietic cells by the upregulation of cytokines. HIV-1 gp120 can stimulate monocytes to release TNF-α, IL-1β, IL-6, and GM-CSF (77, 78). A similar cytokine profile of increased TGF-β1 (51), TNF-α (79), TNF-β (80), and IL-6 (81) levels is seen with exposure to HIV-1 Tat.

It is difficult and impractical to separate the cytokine signals and examine them in isolation from one another as the implications of affecting any one of them can only be analyzed in the context of the milieu in the bone marrow microenvironment. However, generally, in HIV infection, the cytokine pattern results in a polyclonal B-cell stimulation early in disease as a result of heightened Th2 responses. Once HIV viral load increases, the pathogenicity of the infection itself along with the effects of HIV proteins cause a cytokine profile conducive to dysplasia of the marrow and a reduced capacity to support hematopoiesis due to inhibitory signals. In late disease, a decline in Th2 cytokines with further immune dysregulation due to the dwindling T cell population from primary infection results in bone marrow hypoplasia and fibrosis (9).

5. GENE THERAPY FOR HIV

Since the primary sites of HIV infection are the hematopoietic and immune systems, gene therapy targeting these sites for control and eradication of the virus is an active area of study. The approaches entail the use of suicide genes, activated by HIV infection, or by genes rendering cells HIV-resistant by intracellular “anti-HIV” mechanisms. In either case, the target cell is the HSC due to its relative HIV-free status, and its ability to reconstitute the immuno-hematopoietic repertoire (82) with potential for long-term efficacy. The purpose of this section is to provide a brief overview of gene therapeutic approaches in HIV and the use of the CD34+ HSC in this context.

5.1. Strategies for HIV Resistance

Different strategies for anti-HIV gene therapy exist based on antagonizing viral replication at various points in its life cycle. RNA-based inhibitors to viral replication, integration and propagation are not as immunogenic as their protein-based counterparts and can be expressed at high levels without interfering with normal cellular functions (83). They include ribozymes (84-86), anti-sense RNA (87), and RNA decoys (88). Ribozymes and anti-sense RNA are very specific to their respective targets. This can be advantageous as it confers specificity of the interaction and flexibility in that a variety of different messages can be targeted (83-87). Furthermore, ribozymes can cleave their targets diminishing the requirement for abundant expression of anti-sense message. Alternatively, RNA decoys compete for the same binding sites on nascent viral RNA as normal regulatory sequences, thereby preventing transcription and splicing signals required for viral propagation (83, 88). The advantage of RNA decoys lies in the fact that they target highly conserved areas of the genome and therefore are unaffected by subtle variations in the virus. If the sites at which the decoys compete are

altered, the virus is unable to bind the normal regulatory sequences and therefore is already replication deficient (83). Protein-based strategies for HIV resistance depend on the alteration of HIV genes to produce proteins with a transdominant mutant phenotype, which hinder viral replication due to ineffective transcription, splicing, or assembly (83, 89-91). It is not clear at this time which approach, if any, is best for anti-HIV therapy, although all are effective at least *in vitro*. However, one study comparing a few of the different strategies targeting the Rev transcript or protein directly shows that the transdominant mutant protein (Rev M10) confers more anti-HIV activity than an RNA-decoy (Rev Response Element [RRE]-decoy) or a hammerhead ribozyme able to cleave two different transcripts (*tat* and *rev*) (91). Although the protein-based form of therapy may be superior, it is also likely to be more immunogenic (89, 90). Perhaps a more important concept inherent to all of these strategies, is the fact that monocytes, macrophages, and T-cells matured from HSCs transduced with the gene of interest reliably express the respective product after commitment, without altering normal cell function, and are thereby resistant to HIV infection (83-91).

5.2. Methods for Gene Delivery

Retroviral vectors, such as Moloney murine leukemia virus (Mo-MuLV), and adeno-associated viral (AAV) vectors can both be used for insertion of the gene of interest into hematopoietic cells (83, 92). Although generally both prefer the dividing cell for entry, AAV can also integrate into non-dividing cells, such as HSCs (5). Ironically, a promising method for the introduction of genes into the non-cycling HSC, is by the use of lentiviral vectors (in the retrovirus family), of which HIV is a member (5, 84, 92-98). These vectors can be used to target both CD4+ cells *in vivo*, thereby restoring the immune system, as well as the HSC for immuno-hematopoietic reconstitution (5, 93-100).

Typically, for delivery of gene therapy to affected patients, bone marrow is harvested, enriched for CD34+ HSCs, and manipulated *ex vivo*. The HSCs are stimulated with cytokines into cell cycle, which allows for viral vector entry into the cells with the gene of interest. To increase the yield of transfected cells, they can be selected for the presence of the transduced gene (82) prior to reinfusion into the patient. This brings attention to several issues. First, enriching for CD34+ cells per se is not sufficient for isolation of the HSC that is free of infection. A further step of identifying the CD34+ (CD38-), non-cycling HSC is necessary for the introduction of HIV resistance genes into a cell which is virus-free in late disease and capable of immuno-hematopoietic repopulation. This also provides a method of purging the HIV infected cells from the stem cell product. Although this in theory may be optimal, it is likely not realistic, as the length of time required for engraftment and proliferation of the modified HSC will put the patient at increased risk if a myeloablative conditioning regimen is used for an *in vivo* purge of infected cells. If cells are collected in early HIV disease, the progenitors, as well as the HSCs, may not be infected and still amenable to the transfer of HIV resistance genes, allowing for a better reinfusion product (101).

Second, stimulation of the HSC into cycle with cytokines to allow for transduction is problematic as this limits its multipotential capacity (102). Ideally, therefore, viral vectors not requiring cell division, such as AAV or the lentiviridae, are preferred for long-lasting reconstitution of the hematopoietic repertoire bearing HIV-resistance genes (5, 93, 103, 104).

Third, selection for the transduced cells may no longer be necessary as more efficient gene transfer techniques and detection methods show a 20-100% transduction efficiency and no adverse effects on colony formation of the transduced cells (82, 84, 86, 91, 93, 99). This eliminates the need for a marker gene and the extra post-transduction selection and purification steps prior to reinfusion.

Fourth, more recent methods of obtaining a stem cell product include peripheral blood stem cell (PBSC) mobilization (86, 91, 101, 104, 105), which also allows for quicker engraftment, or cord blood collection (85). Both methods are more easily accessible than a bone marrow harvest, are less cumbersome for the donor, and contain more primitive HSCs. Furthermore, patients who are HIV+ can be mobilized with G-CSF safely, without an increase in viral burden or disease flare, and their mobilized product is comparable to that collected from non-infected individuals (86, 91, 101, 104, 105). Moreover, the HSCs from this product can be transfected with HIV resistance genes and their progeny show not only normal colony growth and development, but resistance to HIV upon exposure (86, 91, 101).

With these considerations in mind, trials in gene therapy for HIV disease can be a reality with the appropriate precautions. Infusion of CD4+ lymphocytes transfected *ex vivo* with a transdominant mutant Rev protein reveals a survival advantage compared to control transfected cells in HIV+ patients (106). However, this approach of modifying only the T cell population ignores other hematopoietic reservoirs in the HIV infected patient. In order to attack this issue directly, the viral burden needs to be decreased, infected cells purged, and HSCs harboring HIV resistance genes given back to the patient for immuno-hematopoietic reconstitution.

6. STEM CELL TRANSPLANT IN HIV: CONSIDERATIONS FOR THERAPY AND CURE?

Current therapeutic approaches utilizing highly active antiretroviral therapy (HAART), which usually entail a triple drug therapy regimen to combat HIV infection, are very effective in suppressing HIV viral load in a large portion of the HIV+ population to whom it is available. Although this provides effective suppression for certain patients for an unknown period of time, the resurgence of resistant strains after prolonged treatment cannot be predicted and the necessity for protracted therapy places patients at risk for drug effects and interactions. Gene therapy in HIV disease gives the option of not only effectively treating those patients who are refractory to

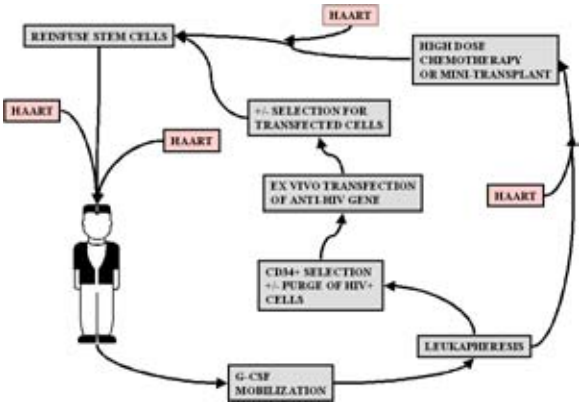

Figure 2: Gene therapy for HIV. As the CD34+ stem cell is virtually free of HIV infection, it can be manipulated *ex vivo* with genes that will not only resist HIV infection, but that will remain in the genome. After stem cell mobilization, the HIV-infected patient would therefore undergo high-dose chemotherapy and stem cell rescue, or, depending on the type of myeloablative therapy used, a series of mini-transplants, with the *ex vivo* manipulated stem cells. See text for greater detail.

treatment or cannot be treated with usual regimens, but also the opportunity to engender cells with HIV resistance as they mature. This can be accomplished with stem cell transplant of genetically modified HSCs.

In considering gene therapy and stem cell transplant for HIV disease, there are several premises with which to start, most of which are described in previous sections:

1. There is a more profound suppression of the hematopoietic repertoire with increased HIV viral burden. This is due to HIV infection of the cells themselves, HIV suppressive proteins, and an altered cytokine milieu caused by HIV.
2. The CD34+ HSC, and HPC in early disease, are spared from HIV infection.
3.CD34+ HSC/HPCs can be safely mobilized from HIV infected individuals, and their progeny produce colonies normally.
4. Using appropriate vectors, HIV-resistance genes can be introduced into the CD34+ HSC/HPC safely, without adverse effects on normal cellular function, and confer resistance to their progeny upon exposure to virus.
5. The bone marrow microenvironment, and in particular, bone marrow stromal elements are likely infected with HIV and act as a reservoir for virus.
6. After stem cell transplant, bone marrow stromal elements may become donor in origin (54), and transduced CD34+ cells are detected for at least 1-3 years after transplant (107, 108).
7. CD34+ cells mobilized form HIV+ patients can be safely stored in liquid nitrogen without losing their susceptibility to retroviral transduction or ability to differentiate (86).
8. HAART can effectively suppress HIV for an indeterminate period of time.
9. At least 10% of the stem cell product reinfused should be phenotypically modified for effective therapy of most inherited and acquired disorders. Without prior myeloablation, competitive repopulation of the stem cells occurs after reinfusion, reducing the amount of transduced stem cells able to engraft effectively (92).
10. Previous attempts at autologous and allogeneic transplants for HIV-related conditions in infected individuals show that the myeloablative regimen is well tolerated, and produces a transient decline in the viral burden, even though the patients died from recurrent lymphoma, AIDS or other infections (86, 109, 110).

Based on these premises, one can envision a method of gene therapy in HIV using a modified stem cell product after a degree of myeloablation of the host tissues (figure 2). This could be in the form of one transplant or a series of "mini-transplants" with more mild conditioning regimens. HAART needs to be utilized throughout the pre-mobilization, mobilization, conditioning and transplant phases to effect a condition of low viral burden, akin to the minimal residual disease (MRD) state in malignant diseases, and, ideally, patients with early disease should be chosen for the procedure. This will ensure the procurement of a product with the lowest viral burden possible, and an appropriate milieu for engraftment. The myeloablative regimen should effect an *in vivo* purge of infected cells in addition to suppressing competition for the graft. Thereafter, transduced HSC/HPC can reconstitute the immuno-hematopoietic repertoire, and perhaps eventually even replace the bone marrow stromal elements. This will allow for effective *in vivo* HIV-resistance and normal hematopoiesis and immune function to be restored, with a possibility for hematologic cure. Other reservoirs for HIV will still remain a problem, unless the disease is caught prior to their infection.

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