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Stem Cell-Based Approaches to Treating HIV Infection

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Abstract

Purpose of Review—Stem cell based strategies for treating HIV infected individuals represent a novel approach toward reconstituting the ravaged immune system with the ultimate aim of clearing the virus from the body. Genetic modification of human hematopoietic stem cells to produce cells that are either resistant to infection, cells that produce lower amounts of infectious virus, or cells that specifically target the immune response against the virus are currently the dominant strategies under development. This review focuses on the understanding of stem cell based approaches that are under investigation and the rationale behind such approaches.

Recent Findings—Significant progress has recently been made utilizing stem cell based approaches to treat HIV infection. Ideally, a successful strategy would result in immune clearance of the virus from the body as well long-term restoration of overall immune responses to successfully combat everyday environmental antigens. Two recent clinical trials illustrate how new advances in stem cell based approaches may propel this field forward to clinical reality: one that demonstrates that large-scale gene therapy trials can be performed in a conventional, reproducible manner; and one that demonstrates the utilization of a multi-pronged approach using lentiviral-based gene therapy vectors. These clinical trials serve as the foundation for the development of other technologies, discussed here, that are currently in preclinical development.

Summary—Recent advances using stem cell based approaches to treat HIV infection have provided the impetus for a renewed and expanded interest in the development of new cell-based strategies to treat HIV infection as well as a variety of other diseases.

Keywords

stem cells; gene therapy; HIV therapy; engineered immunity

Introduction

HIV infection takes a significant toll on the human immune system, wearing it down into numerical and functional oblivion. The advent of suppressive antiretroviral therapy has had a significant effect on slowing this process and on partially restoring aspects of the immune response lost to the effects of HIV-induced pathology. Even the most successful of the

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current therapeutic regimens are incapable of eradicating the virus from the body and do not fully restore functional immunity to the levels seen prior to HIV infection. In this regard, there is an important need for therapeutic strategies that are aimed at enhancing the host's ability to effectively clear the ongoing viral infection and restore the damaged immune system. Recently, there has been increased interest in the development of strategies that utilize human hematopoietic stem cells (HSCs) to treat HIV infection and other AIDS-related diseases [1–6].

Successful stem cell based therapies would ideally have several advantages over conventional pharmacologic approaches. First, stem cells have the ability to reconstitute and produce naïve populations of immune cells for prolonged periods of time. Second, HSCs are amenable to genetic manipulation, thus allowing the addition of exogenous elements to the progenitor cells that protect progeny from direct infection and/or allow them to target a specific antigen. Further, HSC based approaches can allow multilineage hematopoietic development to occur, thus reconstituting multiple arms of the immune system. Finally, due to the fact that HSCs can be derived in an autologous fashion, immune tolerance is likely to be high therefore contributing to successful engraftment and subsequent differentiation into functional mature hematopoietic cells. While HSC transplantation technology and procedures are not exactly new, as they are frequently and successfully used in the treatment of many different types of hematological disorders and malignancies [7], the combination of these with a gene therapy approach represents a novel and potentially powerful treatment strategy for HIV and AIDS-related conditions. The primary purpose of this review is to briefly discuss two distinct, basic approaches in the use of human HSCs to restore the human immune system in HIV disease: one approach that attempts to protect newly formed cells from HIV infection and one approach that attempts to produce immune cells that target HIV infected cells. While each approach currently has many facets, they represent a growing interest in treatments for HIV/AIDS that would, most likely in combination with other therapies, result in overcoming the barriers that are necessary for the body to successfully clear the virus.

Viral Persistence and Immune Failure

In the natural history of HIV infection, the common outcome is the failure of the immune response to clear the virus from the body resulting in a persistent infection for the life of the infected individual. The consequences of this persistent infection are a constant interplay between host immune responses and the virus and its ability to mutate and evolve. The fact that HIV hijacks the immune system itself for its replication has considerable effects on the entire physiology of the affected individual. The damage is done to the human immune response very shortly after acute infection with HIV, particularly in the CD4⁺ T cell compartment in the gut, and increases over time towards the development of AIDS and death [8,9]. A potent, but insufficient, immune response is generated against HIV early following infection that never overcomes the replicative ability of the virus in most individuals [10–12]. This failure is not well understood, but the qualitative and quantitative degrees to which the response is generated are critical components in disease progression and pathogenesis [13–16]. It is critical that a treatment strategy aimed at clearing the virus from infected individuals contributes to the regeneration of immunity that at least partially repairs the damage done by HIV.

Stem Cell Approaches to Treating HIV

A primary therapeutic goal towards treating HIV infection is the augmentation or enhancement of natural immune responses that lead to the direct clearance of the virus from the body. The restoration of a fully functional immune system following HIV infection

would be critical to achieve optimal antiviral responses to clear the virus as well as enable the individual to fully cope with other environmental insults and maintain a better quality of life. The advent of virally suppressive antiretroviral therapy (ART) has revealed that the immune system has a high degree of plasticity and can allow significant cellular reconstitution, albeit not to the levels seen prior to HIV infection [17,18]. In some cases, antiretroviral therapy can result in immune reconstitution inflammatory syndrome (IRIS) in the rebounding immune response, causing significant morbidity and mortality in individuals with low CD4 cell counts prior to the initiation of therapy [19,20]. IRIS is essentially characterized by an out of control immune response against a co-infecting pathogen, most likely due to the direct dysregulation caused by HIV infection and the lack of proper immune control by dysfunctional or depleted immune cells of different leukocytic lineages [19]. This type of “skewed” reconstitution highlights the importance of a strategy that promotes multilineage hematopoietic immune restoration in a balanced way. The use of HSCs represents a potentially powerful therapeutic approach that has the potential to restore total functional immunity to the affected individual.

A successful stem cell-based antiretroviral therapeutic strategy would have to satisfy two major requirements: 1) the approach would have to allow the reconstitution of immune responses that would overcome the barriers necessary to clear the virus from the body, and 2) the newly developed cells would themselves have to be protected from direct infection by HIV, thus preventing them from becoming another reservoir of infected cells. Satisfying these two conditions is not likely to be done by any single new “magic bullet” therapy or approach, but in combination with other new or currently utilized therapeutic strategies; such as combined with the use of suppressive ART. The use of HSCs and their ability to undergo multilineage hematopoiesis and prolonged self-renewal in treated individuals as well as the common clinical usage of bone-marrow transplantation techniques are important starting points in this type of approach. Coupled with the ability to genetically modify these cells, an HSC-based gene therapy approach has recently gained impetus in becoming a viable HIV treatment strategy.

Protecting multilineage hematopoietic cells and leukocytes from infection

Most cells in the human leukocyte lineage express the CD4 molecule at some time during their development as well as HIV coreceptors and are thus susceptible to either direct infection or perturbation. These cell types include “classic” CD4⁺ T-helper cells [21], CD8⁺ T cells [22,23], a subset of hematopoietic progenitor cells [24–26], B cells [27], NK cells [28,29], eosinophils [30], monocytes/ macrophages [31], neutrophils [32], mast cells/ basophils [33], dendritic cells [34] and microglia [35]. Thus, in infected individuals many cell types could potentially serve as viral reservoirs and a successful therapy would allow multilineage protection from infection with particular protection targeted towards T lineage and myeloid lineage cells. HSC based approaches that allow this to occur have been attempted and some are currently under development. Anti-HIV ribozymes, intracellular antibodies, short-hairpin (sh)RNAs, dominant negative proteins, decoy RNAs, and zinc finger nucleases are just a few of the currently applied gene therapy technologies designed to render cells refractory to infection or render them less able to produce progeny viruses [3–5,36–38].

A significant advance toward the clinical implementation of stem cell based HIV therapy is found in a recent study that primarily demonstrated the ability to successfully perform a large- scale phase 2 HSC-based gene therapy trial. In this report, investigators utilized autologous adult HSCs transduced with a retroviral vector containing a *tat-vpr*-specific anti-HIV ribozyme to attempt to produce cells less prone to productive infection[4]**. While vector-containing cells were detected for prolonged periods of time (>100 weeks in most

individuals) and CD4+ T cell counts were higher in the anti-HIV ribozyme treated group versus the placebo group, the effects on viral loads were small. However, the success of this particular study is its report that a stem cell-based approach such as this can be used as a more conventional and reproducible therapeutic approach. Another recent clinical study used a multipronged RNA-based approach that involved a ribozyme targeted to CCR5, a shRNA targeted to tat/rev transcripts, and a TAR region decoy[5]**. This study represents a significant step forward in that it is one of the first reports that utilized lentiviral-based gene therapy vectors capable of genetically modifying both dividing and non-dividing HSCs and are less likely than murine retroviral-based vectors to cause cellular transformation. The authors document the long-lived engraftment and multilineage hematopoiesis of vector-containing and expressing cells. While antiviral efficacy was not assessed, the authors demonstrated the safety of this strategy; which reflects positively on the future potential of a lentiviral-based approach.

Several other approaches are currently under development and are aimed at protecting cells from direct infection by knocking out CCR5 expression through RNA interference or by zinc finger nucleases [2*,3*]. These approaches capitalize on the importance of CCR5 in primary HIV infection and the observations that individuals lacking CCR5 expression are relatively resistant to infection [3,39–41]. There is further suggestion the CCR5 has a key role in targeted therapy in the report of the “German patient”, an HIV-infected individual who, during treatment for acute myeloid leukemia, received a heterologous transplantation of HSCs derived from a homozygous CCR5–/– donor following myeloablation[42]*. This individual subsequently controlled virus replication in the absence of ART. Whether a scenario such as this is reproducible or practical is under debate, however this observation provides insight as to the potential importance of the CCR5 molecule in HIV infection and in targeted therapeutic intervention. Several CCR5–/– homozygous individuals have been reported to be infected with HIV-1 [43–53], suggesting that the protection incurred by the lack of CCR5 is not complete and that the viruses in these individuals can utilize alternative co-receptors for infection. This fact, as well as the ongoing viral evolution in infected individuals receiving stem cell based therapy, could hamper the efficacy of a CCR5-targeted strategy. However, CCR5 knockout or knockdown represents a potentially important approach as is evident by the observation that CCR5 heterozygous individuals display delayed disease progression [53] and CCR5 levels in sooty mangabeys appear to be correlated to the lack of disease progression following infection of this natural host to the simian immunodeficiency virus (SIV)smm strains [54]. Thus, a stem cell based approach to knock-down or knock-out CCR5 expression is one strategy that is currently under large-scale investigation; and, coupled with other strategies to control viral replication, has a strong possibility to have a therapeutic benefit.

Engineered antiviral immunity through stem cells

While an approach that blocks the virus from infecting cells of the immune system would ideally allow the regeneration of immune response against HIV, natural immune responses themselves do not possess the inherent ability to prevent infection or clear the virus from the body. Thus, a new focus has been initiated aimed at directing immunity to specifically target HIV in hopes of overcoming the barriers in clearing the virus from the body. These approaches utilize gene therapy-based technologies on peripheral blood cells, to genetically alter cells to express a receptor or a chimeric molecule that allows them to specifically target a particular viral antigen. Many of these approaches simply try to “re-program” cellular immunity, particularly peripheral T cell responses, to target HIV following ex vivo manipulation and re-infusion back into the autologous body [55–58]. Manipulation of peripheral blood T cells from HIV infected individuals has inherent issues that would be necessary to overcome, including the effects that continuous HIV infection and ex vivo

manipulation have on the functionality and lifespan of peripheral blood cells. In addition, these genetically altered cells would express their endogenous T cell receptors and the expression of the newly introduced receptor can result in cross-receptor pairing which could produce self-reactive T cells. A stem cell based approach may overcome many of these defects by allowing selective developmental mechanisms to occur that can generate large numbers of HIV-specific cells in a renewable, stable fashion that can reconstitute defective natural immune responses against HIV.

One approach currently under investigation is to program B cells resultant from HSCs to express an anti-HIV neutralizing antibody [59*,60*]. While neutralizing antibodies have been shown to confer some protection from infection in animal studies and broadly neutralizing antibodies have been observed in some HIV infected people, protection from a single engineered antibody would be unprecedented. However, engineered antibody responses may be useful in enhancing certain immune responses, particularly in the mucosal and innate immune compartments and a clinical benefit may be obtained in combination with other strategies. The understanding of antibody binding and virus neutralization may be further useful in designing chimeric receptors or single-chain therapeutic antibodies with recognition domains that can be utilized in other approaches that target cellular immunity towards HIV infected cells[1,61,62]. Thus, genetic engineering of HSCs to produce B cells that secrete a neutralizing anti-HIV antibody or engineering HSCs to allow multilineage hematopoiesis of cells expressing a chimeric cellular receptor containing an antibody recognition domain represent one arm of an “engineered immunity” approach utilizing HSCs.

T cell responses are critical in naturally controlling HIV replication, particularly following the acute stage of infection, and in clearing other types of viral infection[11,12,16]. Engineering T cell responses using HSCs genetically modified with a molecularly cloned T cell receptor or chimeric molecule specific to HIV is another approach in attempting to produce antigen-specific T cells. The fundamental difference in this type of approach is that the cells produced from HSCs following “normal” development in the bone marrow and thymus are subjected to normal central tolerance mechanisms and are antigen specific “naïve” cells, and thus lack the issues of ex-vivo manipulation and functional impairment or exhaustion that other peripheral cell modification methods would have. In this regard, in a proof-of-principle study we have recently demonstrated, using a molecularly cloned T cell receptor specific to an HIV-1 Gag epitope in the context of HLA-A*0201, that HSC modified in this capacity can develop into functional, mature HIV specific CD8+ T cells in human thymic tissue that expresses the appropriate restricting HLA-A*0201 molecule [6]. This demonstrates the feasibility of genetically modifying HSCs with a molecularly cloned receptor and is a step towards the understanding and development of directed T cell responses that could eventually lead to clearing HIV infection from the body in a manner similar to natural immune responses to other viruses and pathogens.

Conclusions

In all, stem cell-based approaches aimed towards the treatment of HIV infection have undergone a renaissance lately and are now a major focus of investigation. Two recent clinical studies highlight the feasibility and reproducibility of performing HSC genetic manipulation and transplantation in an expanded clinical setting. Several technologies are currently under development that target HIV either by protecting the cell from infection or by enhancing immune responses against the virus. Several groups have recently reported that human embryonic stem cells (hESC) can differentiate into the major HIV target cells, T cells, dendritic cells, and macrophages [63–68]. As this field progresses and differentiation protocols for these cells become more efficient, totipotent stem cells such as hESC and the

recently reported induced pluripotent stem cells (iPSC) [69–72] may be very useful for gene therapeutic strategies to combat hematopoietic disorders such as HIV disease. It is likely that in the coming years we will see efficacy studies from this next generation of stem cell based approaches that will serve as the basis for further expansion into the use of these strategies in a variety of therapeutic applications for other chronic diseases.

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