A New Anti HIV/AIDS Strategy: Possible Chemical Induction of Endogenous Type 1 Interferon

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Abstract: Aids is characterized by progressive T cell depletion, immune cells dysfunctions and interferon responsiveness that are driven by chronic activation. Antiretroviral therapy (ART), although effective in improving the survival of HIV-1-infected individuals, has not been able to reconstitute the adaptive immunity. However, ART is neither able to eradicate the virus nor has sufficient immune-modulatory effects to control viral infection. This situation points out the dilemma that current HIV therapy can maintain the disease in a resting state, but not eliminate it. We have described the use of novel chemical agents able to restore T-cell survival by inducing cytokines production. More recently, we suggested a complementary therapy based on the chemical induction of endogenous α/β interferon. We suggest that a therapeutic strategy based upon chemical immune restoration associated with type 1 Interferon (IFN-α/β) might represent a mean for HIV cure. This finding may be vital for future therapeutic approaches in AIDS disease and the immune reconstitution. Understanding these process can lead to a range of new therapeutic interventions.

Keywords: HIV, AIDS, type 1 Interferon, therapy, chemical induction.

INTRODUCTION

Since the use of antiretroviral therapy (ART), human immunodeficiency virus (HIV)-related mortality has declined substantially in the developed countries [1]. However, ART has neither the capability to eradicate the virus nor sufficient immune-modulatory effect to control viral function. In treated patients, a persistent albeit decreased level of apoptosis of peripheral blood CD4+ and CD8+ T cells is observed despite long-term viral suppression [2]. In addition, the long-term use of ART is complicated by drug-related toxicities. Moreover, an increasing number of patients with HIV/AIDS cannot use the currently approved anti-HIV drugs, including the reverse transcriptase and protease inhibitors due to the emergence of drug resistance. Many antiviral compounds presently in clinical use have a narrow spectrum of activity and limited therapeutic usefulness. In many parts of the world the cost of the treatment is an important problem in fighting the disease. Exploring new scientific strategies will provide new insights for future therapeutics that aim at viral eradication. Thus, the search for new anti-HIV molecules represents not only an academic challenge but also a necessity for populations in developing countries. It is time to seriously consider alternative therapies aimed at eradicating the infectious virus from patients.

HIV-1 INFECTION CHARACTERISTICS

HIV infection is characterized by progressive CD4+ T cell depletion and immune cells dysfunctions that are driven by chronic immune activation. The virus infects $CD4^+$ cells, inducing the lysis of infected lymphocytes and the subsequent release of virions. However, the direct killing of infected cells cannot by itself account for the progressive immunodeficiency observed in patients with AIDS. HIV-1 infection is also characterized by an impairment of the immunoregulatory network. This remarkable chronic immune activation is observed in HIV-1 infected patients throughout infection, leading to abnormal expression of various cytokines. Thus, the progression of the disease in HIV-1-infected patients is characterized at both the early and the late stages by a marked dysregulation of the immune system. This phenomenon, observed in humans and in susceptible strains of monkeys (e.g., rhesus macaques), is associated with ineffective control of virus replication; accelerated apoptosis and turnover of T and B lymphocytes; increased levels of T cells expressing markers of activation and proliferation (e.g., antigen identified by monoclonal antibody Ki-67); and elevated serum levels of pro-inflammatory cytokines [3]. Consequently, impaired proliferation response (anergy) and increased apoptosis are the hallmarks of the pathogenesis characteristic of HIV-1 infection [4]. Current ART is presumed to exert a positive effect on CD4+ T cell number and immune function. However, this strategy has limitations concerning the restoration of immune cell functions in that immune recovery generally appears incomplete and variable [5]. More importantly, ART can successfully suppress viral replication but it is not enough to eliminate immune activation. Clearly, the development of new treatments that can restore immune functions may offer a much-needed complementary approach therapy.

HIV-1 LATENT INFECTION

The major obstacle to HIV-1 eradication is the establishment of a latent infection which hampers the cure of

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AIDS. In infected individuals, most of the plasma virus in permissive cells such as CD4+ cells is produced by active rounds of replication. This dynamic process leads to the infection of many cells each day, replacing those killed by infection [6]. Some of the circulating virus are produced by stable reservoirs which carry a latent form of the virus. HIV-1 is found integrated into the genome of resting memory T cells. With adequate immune stimulation, these cells can be subsequently activated and produce virus. This low level of viremia persists despite antiretroviral therapy and results in a viral release from stable reservoirs [7]. The establishment of a reservoir of latently infected CD4+ cells corresponds to a normal process of the immune system. The majority of T cells in the body is in resting state, half of the cells are naïve and the rest are memory cells which have responded to antigens. In HIV infection, the virus replicates preferentially in activated CD4+ cells were killed quickly. However, some of the activated cells can be infected as they are coming back to a resting state. As a result, the latent reservoir becomes clinically important since it renders the HIV infection incurable. In addition to CD4+ T lymphocytes, dendritic cells and macrophages are considered reservoirs for HIV-1 infection. Moreover, infection with HIV-1 results in a remarkable loss of cell-functions and an impairment of the immunoregulatory network. This state is characterized by T cell energy which induces cell hyporesponsiveness to many activation signals (antigens) and cytokines [8].

During the past decade, an enormous body of literature has accumulated from studies conducted *in vitro* and *in vivo* that examined the participation of various factors in regulating the activation of HIV-1 expression. Many additional approaches have been suggested in an attempt to bolster immune recovery during ART including *ex vivo* expansion of T cell populations and the use of cytokines to expand T-cells *in vivo*. Therapeutic efforts to eliminate latently HIV-1-infected cells *in vivo* are in their infancy. We have proposed a new approach aimed at enhancing cell proliferation using chemical agents [9-11]. We described the use of novel chemical agents to restore T-cell survival/proliferation in HIV-1 infection [10]. In this way, small molecules like Trifluoperazine at very low concentration (in which the classical effects of the drug are not observed) could restore the proliferation of T lymphocytes originated from AIDS patients. These molecules enhanced T cell proliferation and eliminated latent infected cells upon HIV-1 reactivation. This restored cell response is associated with the production of IL-2 and Interferon [12]. Despite the HIV-1 release the drug inhibited cell mortality and apoptosis. Identification of new compounds with potential effect against viral reservoirs is in progress. It is likely, that elimination of this reservoir by this novel therapeutic approach will be necessary. Nevertheless, viral eradication can be accomplished on condition that inhibition of HIV-1 replication is maintained. Additional therapeutic interventions are therefore required that help inhibit the virus. To that purpose, we suggested a complementary therapy based on the chemical induction of endogenous α/β interferon [13].

HIV-1 AND TYPE 1 INTRFERON

Type 1 Interferons (IFN-α/β) are produced by most cells upon viral infections, have potent antiviral infections, and can provide therapy for some immune disorders. IFNs are induced by viral infections and also produced constitutively at low levels in hematopoietic tissues and have been imputable various roles, particularly immune system [14, 15]. These functions include effects on the proliferation, survival and differentiation of T lymphocytes, the maturation of B-lymphocytes, and the proliferation and differentiation of dendritic cells [16]. IFN α/β can cooperate with numerous T-cell mitogens, including IL-2, IL-4, IL-7, and IL-12 and can contribute to the restoration of the immune system [17] and are important factors in innate immunity, through their enhancement in T cell stimulation [18]. Moreover, IFNs are endowed with multiple biological activities [19]. They are important players in innate immunity, through their antiviral activity against viruses and through their enhancement of T cell stimulation. Type 1 IFNs are considered to be major players for linking innate to adaptive immunity. They enhance adaptive responses by activating dendritic cells (DCs) which are critical antigen-presenting cells for initiating immunity [20]. They promote the expression of costimulatory molecules [21] on human blood monocytes, where type 1 IFN can stimulate differentiation into DCs [22]. More recently, it has been demonstrated that type I IFN must act directly on DCs to induce their maturation into immunostimulatory cells and also is necessary for the generation of a Th1 CD4+ adaptive T cell response [23]. This unappreciated role of IFN-α/β is likely to be an important mechanism that contributes to its therapeutic effects in viral diseases [24].

IFN plays an important role in the treatment and pathogenesis of HIV disease. It has been proven to be particularly effective against HIV-1 replication which affects HIV at several stages of its life cycle [24]. Paradoxically, the appearance of IFN activity in sera of HIV-infected patients is associated with disease progression, suggesting that other cellular factors might regulate the antiretroviral effects of IFN [25]. Recent data in acute simian immunodeficiency virus (SIV)sm infection show a relationship between increasing IFN-α plasma levels and acute viremia, yet IFN-α levels subsequently decreased in spite of sustained viremia [26]. Studies of acute infection in humans have also shown that plasma IFN-α levels transiently increased as observed in SIV infection. Once chronic infection is established, a decrease in IFN-α production has been described in spite of increased accumulation of IFN-α mRNA [27]. This interferon responsiveness probably due to inappropriate activation may be related to the state of anergy observed in AIDS. Evidence has already been developed which demonstrates that AIDS patients are not capable of producing IFNs. In AIDS, patients manifest an abnormal acid-labile circulating IFN-α and are not capable of producing interferon following in-vitro viral induction [25, 28]. This impairment of IFN-α production did not positively correlate with the serum levels of this cytokine that increased at later stages of the disease [29, 30]. It was also reported that cellular responsiveness to exogenous IFN is altered in HIV infection [31, 32] and only higher doses of IFN-α provided protection from apoptosis in cells from AIDS patients [31].

POSSIBLE NEW STRATEGY

More recently, we described the immune-modulatory effects of riluzole (2-amino-6-trifuromethoxybenzothiazole) [33]. Riluzole is characterized by a cationic amphiphilic

structure. As an amphiphilic compound it interacts with membranes and as a cationic molecule it can interact with intracellular targets like DNA (11).

The observation that improvement by Riluzole of the survival of T cells from HIV-infected patients suggests its potential application as an adjuvant in HIV therapy. This cell survival was associated with the anti-apoptotic effect. Riluzole exerts protective effects against the spontaneous apoptosis of both CD4+ and CD8+ T cells derived from HIV-1-infected individuals. The anti-apoptotic effect was maintained during the culture. The Riluzole action might be interpreted as a consequence of the re-entry of a majority of T cells into the G0 cell cycle phase. An unexpected finding emerging from our study is that the enhancement of cell-survival as well as the reduction of apoptosis mediated by Riluzole in cultures was correlated with the synthesis of type 1 IFN (α/β). We have reported that Riluzole can induce the mRNA expression of IFN-α and IFN-β. Only endogenous induced type 1 IFN has this beneficial effect whereas exogenous IFN is not effective. In treated cells, Riluzole can also induce the mRNA expression of toll like receptors TLR3 and TLR7, which were implicated in innate immunity [34]. Single strand (ss) RNA is recognized by TLR7. TLR3 can be activated by double strand (ds) RNA suggesting that viral dsRNA, either genomic or replication intermediate, is critical for the antiviral response. The important mechanism whereby Riluzole modulates the innate immunity is the capacity to induce endogenous (α/β) IFN synthesis. The activation of innate and adaptive responses following acute HIV-1 infection would predict that IFN- could remain functional in spite of continued viral replication. Moreover, Riluzole might improve the IFN system, including different gene transcription activities. IFN-mediated signaling and transcriptional activation of cellular gene expression are best described in the context of Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway proteins. Moreover, different members of the JAK and STAT families have distinct functions in cytokine signaling and additional signaling pathways which are also activated by IFN [34].

In treated cells, Riluzole promotes an increase of viral RNA. Our data clearly indicate that these high levels of viremia do not necessarily lead to cell survival dysfunction. Our approach does not target latently HIV-1 infected cells directly. Nevertheless, the goal is aimed at enhancing cell survival to more rapidly and effectively eliminate latent cells upon reactivation of HIV-1 expression. Establishment of latency is driven by selection of cells that resist viral replication. Then, by maintaining the cell survival associated with a high viral expression, an elimination of latent virus might be possible.

HIV-1 eradication therefore requires therapies based upon both antiretroviral effects and immune activation that could help to restore lymphocyte proliferation, increase IFN response and eliminate the virus-infected cells. Many reports have indicated that endogenous type 1 IFN, either constitutively or induced, plays an important role in host defense [35]. In this regard, endogenous IFN- produced by plasmacytoid dendritic cells (PDC) has been shown to play an important role in controlling HIV infection in human thymus [36], up-regulating host antiviral factors such as APOBEC [37]. Type 1 IFN was described to have a role during expansion and survival of CD8+ T cells, its role in CD4+ cell responses was uncertain. More recently, using polyinosinic polycytidylic acid (poly I:C) as a chemical inducer of IFN, authors found that type 1 IFN was a dominant element of the adjuvant process during CD4+ Th1 immunity *in vivo*. This innate type 1 IFN *in vivo* induced dendritic cells (DCs) to stimulate antigen-specific adaptive immunity [23]. Therefore, the adjuvant action of poly IC requires the endogenous production of type 1 IFN that directly connects antigen presentation by DCs to adaptive immunity.

Clinical studies have shown that patients on ART are treated with Pegylated-IFN-α. Some reports predict that IFN-alpha-2b would mediate viral control in the presence of antiretroviral therapy as already suggested by the decreased HIV titer, observed in HIV/HCV-co-infected subjects, treated with IFN-alpha/Ribavirin [38]. However, the lack of IFN activity in patients might be partly due to inhibitory activity in the fluids. To overcome IFN resistance, increasing endogenous IFN production must be able to reverse the inhibitory effects. Others have shown that endogenous type 1 IFN is responsible for maintaining macrophages [39] and lymphocytes [40] in antiviral state and we consider that endogenous type 1 IFN can be perceived as an essential mediator of innate immunity. In HIV-1 disease, the regulation of intracellular signaling may contribute to unresponsiveness to type I IFN. Actually, monocytes from HIV-1-infected individuals showed diminished responses to IFN, including decreased induction of phosphorylated STAT1 and the interferon-stimulated gene produces myxovirus resistance protein A (MxA) and 2'-5'-oligoadenylate synthetase (OAS) [41]. In this report, the authors suggested that defective monocyte responses to IFN-alpha/beta may play an important role in the pathogenesis of HIV-1 infection.

CONCLUSION

We have described the use of novel chemical agents to restore T-cell survival/proliferation by inducing cytokine production [9-12]. The immunomodulatory effect of Riluzole was ascribed to endogenous type 1 IFN. As possible strategies for manipulation of innate immunity, Riluzole might be used for restoring the innate immunity and inducing type 1 IFN sensitization. It is of interest to emphasize that immune-based strategy in combination with antiviral therapy could control HIV-1 infection. We suggested that a therapeutic strategy based upon endogenous IFN synthesis [33] associated with antiretroviral intervention might represent a means for HIV eradication. Such immune-based strategies should both maintain T-cell activation and enhance the cell functions to clear the HIV-1 infected cells. In this context Riluzole and related molecules might be used as part of a combined antiretroviral-immune therapeutic approach. These results open avenues for therapeutic management of HIV pathological conditions associated with down-regulation of IFN response.

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