JAC antiviral

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Immunosuppressive drugs as an adjuvant to HIV treatment

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Although highly active antiretroviral therapy (HAART) has dramatically changed the epidemiological impact of HIV infection, many problems with currently used antiretroviral therapy have underscored the urgent need for additional therapeutic approaches. Structured treatment interruption trials, which can be considered an immune-based therapy with an autologous virus, have failed to control viral replication in most chronically HIV-1-infected patients. Alternative approaches could be the use of immunosuppressive drugs to enhance the control of viral replication mediated by their immune and antiviral properties. The use of immunosuppressive drugs may reduce the number of activated CD4 cells that support massive virus production and may prevent sequestration of CD4 T cells into lymphoid tissue, which is the place of antigen presentation and productive HIV infection. The strategy of using drugs that interfere with the HIV life-cycle, acting on the target cells of HIV rather than on viral enzymes, offers the advantage of avoiding the development of antiretroviral drug-resistant HIV mutants. However, it is not known if these approaches will clinically benefit long-term infection, by establishing a new immunological set-point that may affect the rate of disease progression. Caution is required when using HAART in combination with cytostatic drugs in HIV-1 infection until their impact and long-term safety have been investigated further in larger clinical trials.

Keywords: hydroxyurea, mycophenolate mofetil, structured treatment interruptions

Introduction

Highly active antiretroviral therapy (HAART) has strikingly diminished the morbidity and mortality of human immunodeficiency virus (HIV) infection.[1] However, drug toxicity and the development of drug resistance are major drawbacks for its long-term treatment. Immune-based therapies, such as immune adjuvants and therapeutic immunizations, are the focus of various HIV researchers. In addition to the significant decrease in the CD4 T cell population, HIV infection is characterized by a profound and continuous state of immune activation manifested by increased turnover of B and T lymphocytes, natural killer cells and a high level of pro-inflammatory cytokines, such as interleukin-7 (IL-7) and tumour necrosis factor-α (TNF-α).[2] Another relevant feature is the elevation of activated CD8+ T cells expressing the DR+/CD38+ phenotype, which is considered a surrogate marker of disease progression.[3] This sustained phenomenon could lead first to an exhaustion of the immune system and second could contribute to the spread of HIV infection. This state of chronic immune activation has been the rationale for the use of immunosuppressive drugs, adjuvant to HAART, such as corticosteroids, hydroxyurea (HU), mycophenolate mofetil (MMF), thalidomide and ciclosporin A.

Failure of structured therapy interruption to enhance an effective specific HIV-1 immunological response

Lessons learned from studies on HIV-infected patients treated since the onset of acute infection, followed by structured treatment interruptions (STI), highlight the major role of the immune system and its feasibility to control viral replication.[4] Re-exposure to viral antigens during treatment interruptions stimulates and boosts the immune system to enhance an HIV-specific response. However, only ~20% of chronically infected patients on STI achieve short-term suppression of viral replication.[5–7] Although a direct correlation between CD8 HIV-specific T cell responses and viral load has been clearly shown in untreated chronic HIV infection, the increases in this response did not correlate with control of viral replication in the majority of STI studies in chronic HIV-1-infected patients. An impairment in HIV-specific CD4 T-helper function, viral escape and/or multifactorial CD8 T cell dysfunction could explain this phenomenon.[8,9] HAART interruption cycles induce sudden antigenic activation with high peaks of viral load, up to a set-point of viral load or higher, which infects new populations of activated CD4 T cells and also could destroy pre-existing antigen-reactive HIV-specific clones.[10] New strategies focusing on avoiding high peaks of viral load after the interruption of HAART are needed.

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Role of hydroxyurea as adjuvant to HAART

Early reports on control of viraemia through treatment interruptions in macaques treated during acute SIV infection with HU as adjuvant therapy encouraged the use of immunosuppressive drugs in other scenarios.[11] This prompted us to hypothesize that use of drugs that inhibit the activation of T lymphocytes during off-HAART cycles limits the infection of target cells, avoiding high peaks of viral replication without blunting HIV-specific immune responses. Although it is well known and clinically proven that HU inhibits the cellular ribonucleotide reductase,[12,13] HU also exerts a cytostatic effect that leads to cell-cycle arrest in the early S phase and a decrease in cellular activation. This last characteristic is the rationale for HU use as adjuvant therapy in patients on an STI trial.

In order to evaluate the clinical immunosuppressive drug effect on viral dynamics, we conducted a randomized controlled study of STI with cycles of HAART or HAART plus HU.[14] Treatment interruptions were scheduled on five cycles of 2 weeks off treatment, but continuing HU during the last two cycles. This schedule allowed us to determine the HU effect, comparing the viral dynamics between cycles on and off HU. First, if HU could inhibit the initial wave of HIV rebound originating from reservoirs—such as quiescent lymphocytes, macrophages or dendritic cells—in which the drug has been shown as an effective monotherapy,[11] the effect of HU should be observed even when HU was stopped. Second, HU could slow the subsequent wave of viral replication driven by activated lymphocytes by virtue of its cytostatic effects. Therefore, control of viral replication should only be observed if HU was maintained.

Although no major differences were observed in neutralizing activity, lymphoproliferative and cytotoxic HIV-specific responses between groups, the increase in the doubling time of viral load rebound was strongly correlated with the lymphoproliferative response as previously described in other STI trials. No major differences were obtained in viral rebound after the first three HAART interruptions. Conversely, when HU was maintained after HAART interruption the viral load was one log lower than previous HU-STI cycles and in addition lower than in the HAART group. This fact strongly supports the hypothesis of the effectiveness of HU as a cytostatic drug and argues against a potential role of HU in reservoirs.

Most importantly, HU significantly increases the proportion of patients who achieve control of viral replication (eight out of nine patients maintained a viral load <5000 copies/mL) after five cycles of STI and after 48 weeks continuously off HAART and despite a baseline viral load of 4.6 log copies/mL.

Role of mycophenolate mofetil as adjuvant to HAART

A well-designed trial proved the possible benefit of immunosuppressor drugs on viral dynamics in early chronic HIV infection. Chapuis *et al.*[15] investigated the *in vitro* and *in vivo* mechanisms by which mycophenolic acid (MPA) and its ester derivate mycophenolate mofetil (MMF, clinical oral drug) suppress HIV infection. MPA selectively inhibits the synthesis of guanosine nucleotides by competing with the inosine monophosphate dehydrogenase. Since there are no alternative enzymatic ways to produce guanosine nucleotides in lymphocytes, MPA clearly exerts a cytostatic and antiviral effect by depletion of this substrate. *In vitro* data showed that MPA inhibited proliferation of activated T cells, especially in those with intermediate and low CD4 expression, driven through apoptosis and cellular death even in the presence of IL-2. These data were also confirmed in a pilot study of patients treated with an abacavir and amprenavir regimen and randomized with or without MMF. In the MMF group, a substantial reduction in the pool of dividing CD4 and CD8 lymphocytes (ki67) was observed. Also, MMF might exert an effect on the pool of resting latent-infected CD4 cells. The authors observed that HAART plus MMF reduced the ability to isolate virus from the CD4 cell population. MMF had no effect on resting cells, and thus it did not affect directly the size of this pool. However, once these cells are activated in the presence of MMF, it could induce apoptosis and cell death.[14]

We conducted a randomized trial to evaluate the effect of MMF on plasma and tonsillar tissue viral load and on immune response during and after an STI study.[16] Patients treated for at least 1 year with an abacavir-containing regimen were randomized to receive or not MMF with HAART for 4 months before treatment interruptions. We hypothesized that MMF could affect viral rebound, especially in MMF-treated patients whose T cell proliferation capacity significantly diminished.

A multiple *in vitro* assay after the MMF dose was used to evaluate the capacity of the patient sera to inhibit the proliferation of a T cell line. It was observed that patients treated with MMF had a remarkable reduction in the size of dividing CD4 T cells and also in viral setpoint after HAART interruption. This effect was mainly observed in those patients who maintained an inhibitory capacity of lymphocyte proliferation (>60%) for at least >4 h after MMF.

Other scenarios

The results of a pilot study on acute HIV-infected patients treated with HAART and short-term ciclosporin A are interesting and encouraging.[17] The rationale for its use was to suppress rapidly the heightened state of cellular activation supporting massive HIV replication. These high levels of viral replication could probably produce clonal exhaustion of HIV-specific CD8 lymphocytes. Although ciclosporin A interfered in the process of Gag HIV proteins, its main effect is probably inhibition of T cell proliferation and differentiation. Clinically, ciclosporin A restored normal CD4 T cells levels early, both in percentage and absolute numbers, and also increased and sustained the proportion of HIV-specific interferon-γ-secreting CD4 T cells.

In another setting, MMF was used as part of salvage therapy and added as a single drug to a rescue-HAART regimen containing abacavir in heavily pre-treated patients harbouring multiple drug mutations.[18] An important viral load reduction (>0.5 log) was obtained in those patients in whom the carbovir (the active antiviral metabolite of abacavir) deoxyguanosine triphosphate ratio was increased due to the inhibition of inosine monophosphate dehydrogenase and depletion of guanosine nucleotides.

Conclusions

Although HAART has dramatically changed the epidemiological impact of HIV infection, the large list of long-term side effects, the eventual development of resistance and the need for long-term compliance have underscored the urgent necessity for additional therapeutic goals and approaches. In addition, the establishment in the early stages of HIV infection of a stable latent reservoir of replication-competent virus cells, primarily in lymphoid tissue, and the ongoing residual viral replication make eradication a non-achievable goal.

Recent data suggest that initiation of HAART during acute infection is associated with long-term control of virus replication after

JAC antiviral

therapy discontinuation, due to preservation of HIV-specific T cell clones.[4] The use of ciclosporin A and probably other immunosuppressive drugs reduced the number of activated CD4 cells that support massive virus production, and may prevent sequestration of CD4 T cells into lymphoid tissue, which is the place of antigen presentation and productive HIV infection. This could have an impact on the milieu of quiescent T cells containing competent replicative viruses. However, it is not known whether—by establishing a new immunological set-point that may affect the rate of disease progression—these approaches will clinically benefit long-term infection,.

In the chronic stage of infection, the STI strategy allows us to control viral replication in ~20% of patients. However, the use of HU and MMF showed the significant improvement in viral dynamics after HAART interruption mainly because of cytostatic and immune properties which did not have a deleterious effect on HIV-specific responses. This approach could also have an indirect impact on the latent reservoir pool, strikingly diminishing the pool of activated T cells. It was demonstrated that MMF reduced the isolation of the virus from the T cell peripheral pool and also from lymphoid tissue, decreasing the release rate of virus from reservoirs. Studies focusing on the correct measure of viral load in reservoirs are needed.

Optimal candidates, exact drug, dosage, duration of therapy and the optimum time to initiate it within the natural course of infection are questions that need to be answered in the context of large clinical trials. Although no major drug toxicity was reported in trials using different drugs, the long-term safety in terms of opportunistic infections and development of lymphoproliferative diseases are a major concern.

The strategy for using drugs that interfere with the HIV life-cycle, acting on the target cells of HIV rather than on viral enzymes, offers the advantage of avoiding the development of antiretroviral drug-resistant HIV mutants. Caution is required when combining HAART and cytostatic drugs in HIV-1 infection until the impact and long-term safety have been further investigated in larger clinical trials.

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