A controlled trial of granulocyte macrophage-colony stimulating factor during interruption of HAART

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Objectives: To explore the effect of granulocyte macrophage colony stimulating factor (GM-CSF) on viral load and CD4 cell count during interruption of highly active antiretroviral therapy (HAART).

Methods: Patients on effective HAART (CD4 cell count $> 400 \times 10^6$/l; viral load < 50 HIV RNA copies/ml) were randomized to one of two groups: 12 weeks' treatment interruption plus, during the first 4 weeks, 300 μg GM-CSF (Leucomax-Novartis) by subcutaneous injection three times weekly (GM-CSF group); 12 weeks' scheduled treatment interruption (STI-only group). Viral load, CD4 cell count, clinical events and side effects of treatment were monitored.

Results: Thirty-three patients, 15 in the GM-CSF group and 18 in the STI-only group, were evaluated according to the intention-to-treat principle. The two groups were well matched with regard to pre-HAART viral loads and CD4 cell counts. During STI, viraemia was approximately two to three times lower in the group receiving GM-CSF (max 4.97 versus 5.45 in STI-only group; $P = 0.03$). Fifteen out of 17 patients in the STI-only group showed a decrease in their CD4 cell count between weeks 0 and 4 (median decrease 231×10^6 cells/l; $P < 0.001$); there was no such tendency in the GM-CSF group ($P =$ non-significant when comparing CD4 cell counts at weeks 0 and 4). The median CD4 cell AUC (area under the curve) from week 0 to week 12 was higher in the GM-CSF group (9166 cells·week) than in patients without GM-CSF (7257), $P = 0.02$. GM-CSF produced local reactions in 88% of patients, and generalized symptoms such as fever, back pain or headache in 82% of patients. Seventy-six percent of patients completed the planned course of 12 injections.

Conclusions: The administration of GM-CSF blunted the viral rebound following interruption of HAART, and largely prevented a decrease of CD4 cell counts during a 12-weeks-treatment interruption. A better understanding of the underlying mechanism(s) may help to identify synergistic treatment targets and improved administration protocols to enhance control of chronic HIV infection.

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Introduction

Highly active antiretroviral therapy (HAART) has revolutionized treatment of HIV infection and has decreased AIDS-related mortality and morbidity by more than 80% [1]. However, problems related to long-term HAART are numerous, such as side effects, lack of compliance and virologic failure due to resistance. For all these reasons, alternatives to continued drug therapy are desirable [2].

In a previous trial, Swiss Spanish Intermittent Therapy Trial (SSITT1), patients were subjected to 2 weeks of treatment interruption, followed by 8 weeks of treatment [3]. After four such cycles, treatment was permanently interrupted. Virus rebound occurred in almost all patients, but then spontaneously declined. However, only 17% of patients stabilized their viral load at levels < 5000 HIV RNA copies/ml [4–6]. The results suggest that additional measures are necessary in order to decrease the viral rebound during treatment interruption. Cytokines appear to be promising candidates. Interleukin (IL) 2 has been tested extensively. At a price of considerable side effects it increases CD4 lymphocyte counts when used in combination with HAART, compared to HAART alone [7–11]. However, after interruption of therapy, the viral rebound does not seem to be influenced by IL-2 [12,13]. Other candidate cytokines such as IL-12 [14] or CD40 ligand [15–17] are either too toxic or not yet available for use in humans.

Recombinant human granulocyte macrophage-colony stimulating factor (GM-CSF) was developed during the early 1990s for treatment of chemotherapy-induced neutropenia [18]. Extensive use has defined its profile of side effects and safety in humans is well established.

Several studies have suggested that GM-CSF may stimulate the immune response to various antigens. Pioneering studies by Dranoff *et al.* showed that immunogenic B16 mouse melanomas produced GM-CSF, in contrast with non-immunogenic melanomas which could, however, be rendered immunogenic by GM-CSF transformation vectors [19]. Injection of GM-CSF together with antigen increased the immune response to tetanus toxoid in rats [20] and to hepatitis B vaccine in humans [21].

The possible use of GM-CSF to bolster immune response has been mainly pursued for immunization against cancer. Phase II studies in melanoma, hypernephroma, prostate cancer and other tumours are in progress. Analogy to cancer vaccinology should not be pushed too far, however; in particular, it is not clear whether the local delivery of antigen together with GM-CSF has the same immunologic effect as subcutaneous administration of GM-CSF during rebound of viraemia [22,23].

The effect of GM-CSF on HIV has been explored both *in vitro* [24–27] and *in vivo* [28,29]. *In vitro,* some studies have found that GM-CSF stimulates viral production [26,27], whereas other studies have found the opposite [25].

In patients, GM-CSF has been used to treat with advanced HIV infection, in combination with antiretroviral therapy [28–30]. In a large prospective double-blind trial, there were indications that GM-CSF might enhance control of viraemia [29]. The drug proved to be safe and well tolerated.

In this trial, we explored the effect of GM-CSF on the rebound of viral load during interruptions of HAART.

Patients and methods

In order to participate in the trial, patients needed to be on antiretroviral treatment, with undetectable viral load for at least 6 months, with a viral load < 50 HIV RNA copies/ml, as measured by the Roche HIV Monitor test version 1.5 (Roche Diagnostics, Rotkreuz, Switzerland), a CD4 cell count $> 400 \times 10^6$/l in the month preceding inclusion, and no treatment with non-nucleoside reverse transcriptase inhibitors (NNRTI) in the month preceding inclusion. Randomization was stratified according to viral load pre-HAART ($< 15\,000$, $15\,000$–$50\,000$ and $> 50\,000$ HIV RNA copies/ml). Patients were randomized to one of two groups: (i) the GM-CSF group stopped HAART during 12 weeks and received GM-CSF (Leucomax-Novartis) during the first 4 weeks, 300 μg three times weekly subcutaneously; (ii) the scheduled treatment interruption (STI)-only group interrupted HAART for 12 weeks without receiving GM-CSF.

HAART was withheld unless one viral load was $> 500\,000$, or two viral loads were between 100 000 and 500 000, or three viral loads between 50 000 and 100 000 HIV RNA copies/ml. Treatment was also reintroduced if symptoms suggestive of the acute retroviral syndrome occurred in a patient with a viral load $> 100\,000$ HIV RNA copies/ml, or if the CD4 cell count fell $< 350 \times 10^6$/l.

Groups were compared at various time points, using means with t tests after log transformation of the values

of viraemia; and medians with non-parametric Mann–Whitney tests for CD4 cell counts. CD4 analysis inside groups used the non-parametric paired Wilcoxon test. Areas under the curve (AUC) were calculated from all values obtained between weeks 0 and 12. In patients who started treatment again before week 12, the last value without treatment was carried forward.

Results

Patients

Thirty-seven patients were randomized. The results of four of these were not analysed for the following reasons: three refused the randomization assignment or treatment interruption after randomization, and one did not fulfil the study entry criteria (viral load > 50 HIV RNA copies/ml on day 0). Of the remaining 33 patients, 15 stopped treatment and received GM-CSF, whereas 18 discontinued treatment without GM-CSF. The two groups were well matched with regard to pre-HAART viral load: in the GM-CSF group mean viral load was 4.56 $\log_{10}$ copies/ml (25th, 50th and 75th percentiles were 4, 4.32, and 5.3 $\log_{10}$, respectively), in the STI-only group mean viral load was 4.8 (percentiles: 4.2, 4.86, 5.5 $\log_{10}$) ($P = 0.4$). There was no statistical difference in pre-HAART CD4 cell count: median 325×10^6/l (percentiles: 240, 325, 537×10^6/l) in the GM-CSF group and 337×10^6/l (percentiles: 238, 337, 442×10^6/l) in the STI-only group ($P = 0.87$), or in median CD4 cell count at day 0: respectively 890×10^6/l (percentiles: 645, 890, 929×10^6/l) and 720×10^6/l (percentiles: 592, 720, 924×10^6/l) ($P = 0.26$). Patients were treated with conventional HAART including two nucleoside reverse transcriptase inhibitors (NRTI) plus one protease inhibitor (PI; 61%), or three NRTI including abacavir (33%); two patients (6%), one in each group, were treated with only two NRTI.

Plasma HIV RNA response

The viral load increased, peaked after about 6 weeks, and fell spontaneously in both groups. The maximum $\log_{10}$ viral load reached a mean of 4.97 in the GM-CSF group and 5.45 in the STI-only group ($P = 0.03$, t test). Mean AUC of the viral loads (missing values were carried forward) between week 0 and week 12 were 47.77 $\log_{10}$ copies·week in the GM-CSF group, and 51.88 log copies·week in the STI-only group ($P = 0.07$) (Table 1).

CD4 count response

From week 0 to week 4, CD4 cell counts fell in the STI-only group [medians 720×10^6/l at week 0 (in percentage of lymphocytes, 34%) and 537 (25%) at week 4, median decrease of 231×10^6/l; $P < 0.001$ for all comparisons by Wilcoxon test]. There was no such tendency in the GM-CSF group when comparing CD4 cell counts at week 0 (median, 890×10^6/l; 37%) and week 4 (median, 792×10^6/l; 35%; $P = 0.6$). Of the 17 patients in the STI-only group 15 (88%) had a decrease of CD4 cell counts between week 0 and 4, compared to 47% (7/15) of patients in GM-CSF group ($P = 0.018$, bilateral chi-square with Fischer's correction) (Fig. 1). The difference in CD4 cell counts between the two groups was statistically significant at week 4 ($P = 0.001$) but partly disappeared by week 12 with a median of 465×10^6 CD4 cells/l in the STI-only group compared to 663×10^6/l in the GM-CSF group ($P = 0.078$).

Median AUC (weeks 0–12) were 9166 cells·week for the GM-CSF group and 7257 in the STI-only group ($P = 0.02$).

Table 1. Viral load and CD4 cell count responses in the granulocyte macrophage-colony stimulating factor (GM-CSF) and scheduled treatment interruption (STI)-only groups.

	GM-CSF group	STI-only group	P
Mean plasma viral load ($\log_{10}$ copies/ml)			
Pre-HAART	4.56	4.8	0.4
Maximal	4.97	5.45	0.03
AUC[a] (week 0–12)	45.77	51.88	0.07
Median CD4 cell count ($\times 10^6$/l)			
Pre-HAART	325	337	0.87
Week 0 (baseline)	890	720	0.26
AUC[b] (week 0–12)	9166	7257	0.02

[a]With last value carried forward, mean in $\log_{10}$ (copies)·week. [b]With last value carried forward, median in cell·week. HAART, Highly active antiretroviral therapy; AUC, area under the curve.

Clinical events, treatment

During week 0–12, two patients in the GM-CSF group and six patients in the STI-only group were retreated because of high viral load. Three patients, all in the STI-only group, had signs or symptoms of acute retroviral syndrome. After week 12, a further six

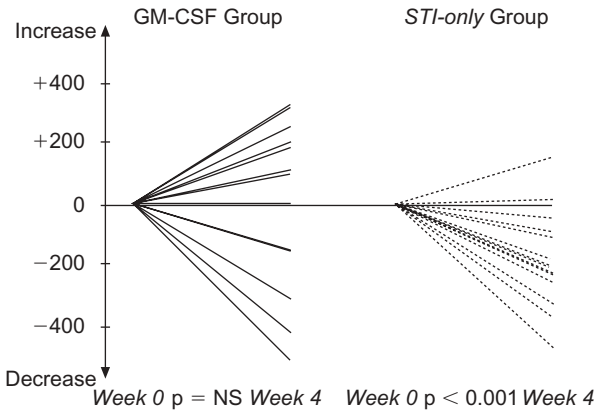

Fig. 1. Changes in CD4 cell count between weeks 0 and 4.

patients started treatment again, two because of high viral load (both in STI-only group), two (one in each group) because of low CD4 cell count (including one who was lost to follow-up for several months and presented with *Pneumocystis carinii* pneumonia), one because of skin lesions associated with acute hepatitis B (STI-only group), and one by patient choice (GM-CSF group). In all 14 of these patients restarting HAART was followed by decline of viraemia within 3 months to $<$ 400 HIV RNA copies/ml.

GM-CSF side effects

Toxicity analysis is based on 17 patients using GM-CSF (including the 15 analysed above plus one who did not fulfil the entry criteria, plus one who never interrupted treatment). Most of these patients experienced side effects. Eighty-eight per cent of patients (15/17) complained of local pain, redness or swelling at injection sites (including two patients with regional extension). Eighty-two per cent of patients (14/17) had general reactions including two who presented serious adverse reactions after the first injection (one with diarrhoea, malaise, and hypotension and one with pharyngitis, periorbital oedema and back pain). Four patients ceased GM-CSF before the planned 12 injections because of general and/or local side effects.

Discussion

This pilot study showed that GM-CSF had a favourable effect on CD4 cell counts during treatment interruption which were evident during and shortly after its administration during weeks 1–4 of STI, but appeared to wane by week 12. Regarding viral load, results also tended to favour the GM-CSF group. However, the study was small, and not all results reached statistical significance.

It is important to point out some limitations of our study. These were patients who had received effective HAART for at least 6 months before entering the trial. They had not experienced virologic failure. Other patient populations may not fare as well and might, for instance, develop resistance to treatment.

It is uncertain whether the favourable influence of GM-CSF on HIV surrogate markers can be translated into clinical benefit. This is mainly because of the high incidence of side effects that we observed. This is in marked contrast with the findings in the large study by Angel *et al.* [29], with only 25% injection site reactions, most of which were mild. Patients in that study were severely immunosuppressed, in contrast with the patients in our study, and the GM-CSF preparation was not the same brand; this may explain some of the differences. These results raise the question of whether or not GM-CSF would be effective and tolerated when administered for longer periods, and possibly at lower doses and/or less frequently.

The mechanism of the effect of GM-CSF is presently unknown. Contrary to other agents which blunt viral rebound during treatment interruptions, such as hydroxyurea [30,31], GM-CSF has many different effects on almost all cells involved in the immune response, including neutrophils [32], lymphocytes [33], macrophages, monocytes [34], dendritic cells [35], endothelial cells, and natural killer cells [36]. As noted in the introduction, GM-CSF is widely used as immune adjuvant in conjunction with cancer vaccines. However, it is usually combined with antigen in a localized injection; this is different from the situation in the present trial, where generalized antigenaemia is combined with subcutaneous injections of GM-CSF. Apart from stimulating the anti-HIV immune response, including neutralizing antibodies and cell mediated lymphocytotoxic immunity, GM-CSF also decreases the expression of the HIV co-receptor CCR5 on lymphocytes [37]. CCR5 expression correlates with HIV viraemia in HIV-positive children [38]. Further research will have to show which, if any, of these mechanisms explain the present findings. A better understanding of the underlying mechanisms may also help to identify new treatment targets which could extend the beneficial, but limited effects of GM-CSF on HIV-specific immunological control.

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Appendix

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