Imperial College London

POSTER # 495

Effects of Recombinant Human Growth Hormone on HIV-1-Specific T-Cell Responses, Thymic Output and Proviral DNA in Patients on ART: 48-Week Follow-up

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ABSTRACT #H-109

Background: Immunotherapeutic strategies in treated chronic HIV-1 infection require induction of virus-specific CD4 T cells and subsequent activation and maintenance of specific memory CD8 T cells. Concomitant daily administration of recombinant human growth hormone (rhGH) therapy with effective ART was used in chronically infected patients in an attempt to reconstitute HIV-1-specific CD4 and CD8 T-cell responses. **Methods:** Twelve individuals with chronic HIV-1 infection on effective ART (VL BDL, CD4 >200 cells/ul) were enrolled in this randomised, double-blind, placebo-controlled study to receive rhGH therapy (4mg/day subcutaneously). We assessed HIV-1-specific proliferative CD4 and interferon (IFN)-γ-producing CD8 T-cell responses, TREC levels and quantified proviral HIV-1 DNA: at baseline; after 12 weeks of rhGH therapy; at 24 weeks after randomisation into three groups [placebo weeks 12-24 (Group 1), alternate-day dosing weeks 12-24 (Group 2), and twice-per-week dosing weeks 12-24 (Group 3)]; and at 48 weeks after all patients had received ART alone for the final 24 weeks. **Results:** We found significant increases in both proliferative CD4 (evaluated with both recombinant and whole-HIV-1 antigen) and IFN-γ-producing CD8 (evaluated with both rVV and peptide pools) HIV-1-specific T-cell responses after daily administrations of rhGH. Following subsequent randomisation to different dosing regimens, HIV-1-specific proliferative CD4 T-cell responses declined in those receiving less frequent dosing of rhGH, while virus-specific IFN-γ-producing CD8 T-cell responses were maintained for longer periods of time. Whilst viral load, CD4 and CD8 T cell counts remained unchanged, there was significant enhancement in T-cell maturation and differentiation. TREC levels and cell-associated HIV-1 DNA were stable in most patients. **Conclusions:** The implication of these data is that concomitant administration of rhGH with effective ART results in a dose-dependent reversal of both the CD4 and the CD8 T lymphocyte dysfunction commonly observed in HIV-1-positive patients. Such immune-based therapeutic strategies in treated chronic HIV-1 infection may enable the induction of virus-specific CD4 T cells essential for the subsequent kick-start and expansion of specific CD8 T cells.

INDUCTION OF HIV-1-SPECIFIC T-CELL RESPONSES WITH ADMINISTRATION OF rhGH IN THE PRESENCE OF ART

HIV-1-specific CD4+ helper (HTL) and CD8+ cytotoxic (CTL) T lymphocyte responses have been shown to inversely correlate with viral replication, disease progression and even protection from productive infection in HIV-1 infected and seronegative individuals. Immunomodulation with rhGH together with effective ART may boost the missing HIV-1-specific responses in chronic HIV-1 infection. PBMC from twelve HIV-1 infected individuals with lipoatrophy on long-term successful ART receiving rhGH (Serostim, Serono International, Geneva, Switzerland), were assessed for HTL proliferation and CTL IFNγ production.

Aims:
1. Increase HIV-1-specific immune responses
2. Successful immunotherapeutic intervention
3. Purge infectious virus from reservoirs
4. Discontinue ART

Recombinant human growth hormone administration:

Given at 4mg/day for 12 weeks to 12 HIV-1+ chronically infected individuals receiving successful ART

12-24 weeks

Randomisation → Placebo / Alternate day dosing / Twice weekly dosing

24-48 weeks-back on effective ART alone (no immunotherapy)

Samples were collected at baseline and weeks 12, 24 and 48 of the study.

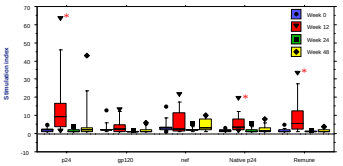
TABLE 1

Patient characteristics at baseline, weeks 12, 24 and 48

		Baseline			Week 12			Week 24			Week 48		
Patient*	Age (years)	CD4 Cells/µl	CD8 Cells/µl	HIV-1 RNA copies/ml	CD4 Cells/µl	CD8 Cells/µl	HIV-1 RNA copies/ml	CD4 Cells/µl	CD8 Cells/µl	HIV-1 RNA copies/ml	CD4 Cells/µl	CD8 Cells/µl	HIV-1 RNA copies/ml
1 B	40	823	1239	U/D	714	1218	U/D	888	1027	U/D	745	956	U/D
2 C	32	436	555	U/D	422	735	U/D	456	667	U/D	418	567	U/D
3 C	38	636	1235	1555	636	1209	5017	676	1179	2027	518	737	2788
4 B	39	684	1613	U/D	425	1137	U/D	371	899	U/D	415	980	U/D
5 A	45	667	553	U/D	697	680	U/D	647	559	U/D	540	557	U/D
6 A	58	320	421	U/D	431	837	U/D	393	917	U/D	364	750	U/D
7 C	41	257	884	U/D	269	747	U/D	375	986	U/D	443	954	U/D
8 B	40	622	1602	17089	435	776	U/D	609	1202	U/D	577	942	U/D
9 B	53	211	1074	U/D	217	1336	U/D	844	1369	U/D	241	884	U/D
10 C	40	293	715	U/D	262	791	U/D	286	708	U/D	ND	ND	ND
11 A	52	374	1388	U/D	543	1624	U/D	713	2357	U/D	599	1364	U/D
12 A	43	498	1061	U/D	375	980	U/D	421	988	U/D	341	799	U/D
Mean/sum	43.12	478.6546	10200.1116	17644.1414	4529.48	1006.486	4424.413	5241.05	10780.147	2250.100	4739.42	9281.108	2788.2149

FIGURE 1

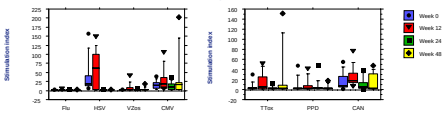
Immunotherapy with rhGH in the presence of effective ART induced a significant (p<0.005)* increase in HIV-1-specific CD4 T-cell responses.



At baseline lack of proliferative CD4 virus-specific responses to any HIV-1 antigen were noted in 11 of 12 individuals. Daily rhGH increases both gag-specific and whole HIV-1 antigen (Remune)-specific CD4 T-cell responses over a 12-week period in 9 of 12 patients with chronic infection on ART (Fig 1). Benefits are not maintained with less frequent dosing.

FIGURE 2

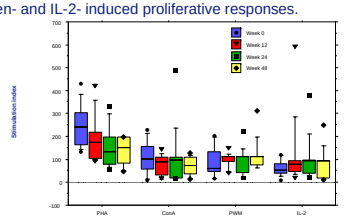
Immunotherapy with rhGH in the presence of ART had no effect on CD4 T-cell responses to other viral and recall antigens.



Daily rhGH did not affect non-HIV-1-specific CD4 T-cell responses over a 12-week period in ART treated chronic infection (Fig 2). Consistent responses to other viral and recall antigens were maintained throughout the course of study. However, significantly higher (p<0.05) responses to Herpes simplex virus (HSV) antigens were observed at baseline, which increased at week 12 and disappeared thereafter (and correlated with clinical manifestations).

FIGURE 3

Immunotherapy with rhGH in the presence of effective ART had no significant effect on mitogen- and IL-2- induced proliferative responses.

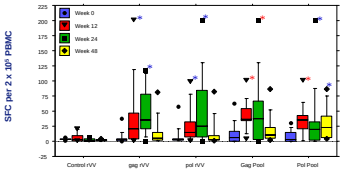


Daily rhGH did not affect the mitogen and IL-2 induced T-cell responses over a 12-week period in ART treated chronic infection (Fig 3). Consistent responses are maintained throughout the course of study. The peak of the robust mitogen induced proliferative responses at week 0 and weeks 12, 24 and 48 might have been missed (since these are post-optimal after 5 days in culture). See PHA IFNγ ELISPOT data below (Fig 5).

INDUCTION OF HIV-1-SPECIFIC CD8 T-CELL RESPONSES WITH CONCOMITANT ADMINISTRATION OF rhGH AND EFFECTIVE ART

Immunotherapy with rhGH in the presence of ART induced a significant increase in HIV-1-specific CD8 T-cell responses evaluated with rVV constructs (p<0.05)* and peptide pools (p<0.005)* of gag or pol proteins in IFNγ ELISPOT assays (Fig 4).

FIGURE 4

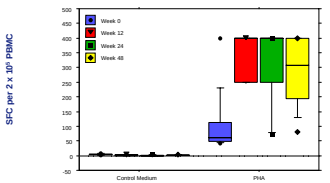


Virus-specific CD8 T-cell responses were maintained at week 24 regardless of which arm the patients were in. At week 48 CD8 T cell responses declined (except those directed at peptide pools of pol) (Fig 4).

rhGH INCREASES TOTAL MITOGEN-SPECIFIC IFNγ-PRODUCING T-CELL RESPONSES IN A 24hr ELISPOT ASSAY

Although we could not detect any difference by assessing mitogen-specific proliferation ([3H]-thymidine incorporation in a 5-day assay; Fig 3), immunotherapy with rhGH in the presence of effective ART induced a significant (p<0.005) increase in PHA responses evaluated with IFNγ ELISPOT assays (Fig 5).

FIGURE 5



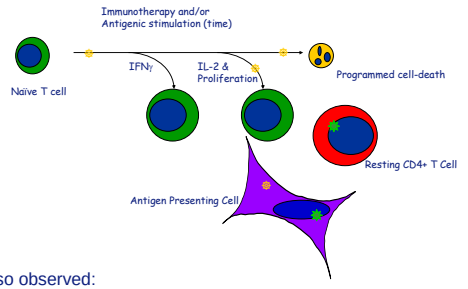
The increased PHA responses were maintained throughout the course of study (Fig 5).

ACKNOWLEDGEMENTS

The authors thank patients and staff at Chelsea & Westminster Hospital who participated in this study. This work was supported by the Wellcome Trust (Grant no. 058700) and EU Programme (Grant no. LSHP-CT-2004-503487) .

SUMMARY & CONCLUSIONS

1. Strong HIV-1-specific CD4 and CD8 T-cell responses are augmented by daily administration of rhGH over the 12 week period in HIV-1+ individuals on successful ART.
2. CD4 T-cell responses to other viral and recall antigens, mitogens and IL-2 were not affected (except for anti-HSV responses which concurred with clinical symptoms).
3. Randomisation (into placebo, alternate day dosing or twice weekly dosing) instigated at week 12, showed that HIV-1-specific CD8 T-cell responses were maintained at week 24 regardless of randomisation, despite the disappearance of HIV-1-specific CD4 T-cell responses.
4. At week 48, back on ART alone (no immunotherapy) for the final 24 weeks, HIV-1-specific CD8 T-cell responses declined (except for anti-pol). HIV-1-specific CD4 T-cell responses remained undetectable.
5. Disappearance of CD4 responses suggests that cells may require stronger and/or continuous signals from GH to provide continuous help.
6. CD8 responses may be maintained for a limited period without CD4 T cell help, but actually decline following withdrawal of antigenic stimulus.
7. Whether rhGH may be purging HIV-1 from reservoirs (which primes the newly formed T cells), whilst effective ART is preventing de novo infection remains to be seen.
8. Feedback mechanisms between hormones and cytokines may also be operational.
9. We provide novel data inferring that concomitant administration of rhGH with effective ART may reverse defects exerted on the immune system by HIV-1 (see diagram below).



We also observed:

- An increase in naïve CD4 T cells, although TREC and proviral DNA levels remained unchanged (data not shown).
- A significant increase in memory/effector CD8 T cells[1].
- Enhanced NK cell activity[2].

REFERENCES

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