

USE OF DELIPIDATED SIV TO AUGMENT SIV SPECIFIC CD4⁺ AND CD8⁺ T CELL MEMORY RESPONSES IN MICE - A MODEL FOR A NEW THERAPEUTIC VACCINATION STRATEGY AGAINST HIV INFECTION

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ABSTRACT

Background: Novel strategies need to be identified and studied for the immune reconstitution of HIV infected humans to supplement HAART. Current chemotherapeutic strategies, while highly effective, are not without significant side effects, and are associated with increased risk of selection for multi-drug resistant HIV. Such expensive chemotherapeutic strategies are also of limited value in third world countries which share the brunt of this devastating epidemic. We have devised and optimized delipidation formulations which are highly effective in the removal of lipids from SIV preparations *in vitro*. Such optimized delipidation is not only effective in reducing viral infectivity but also appears to retain >90% of the major proteins (SIV Env, Gag, Pol, Tat) of SIV-mac251.

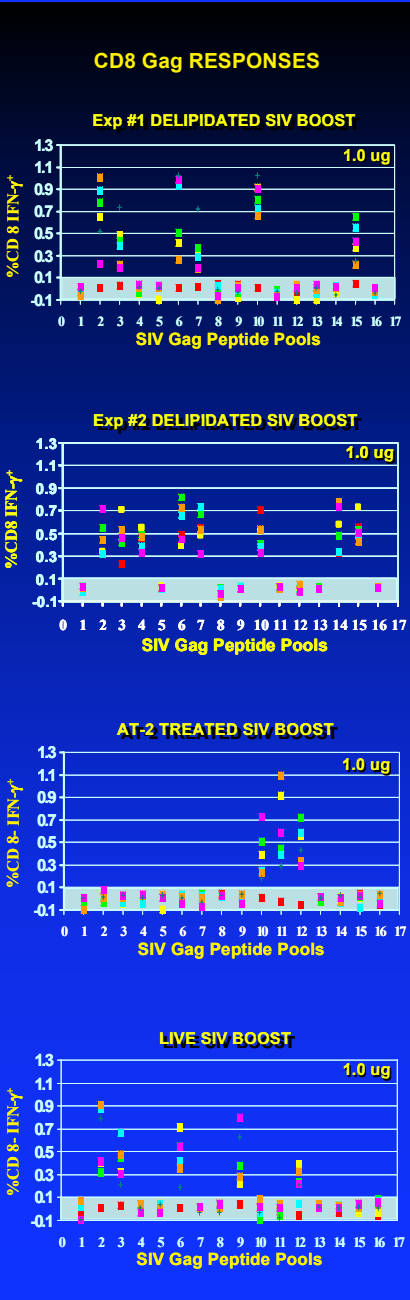
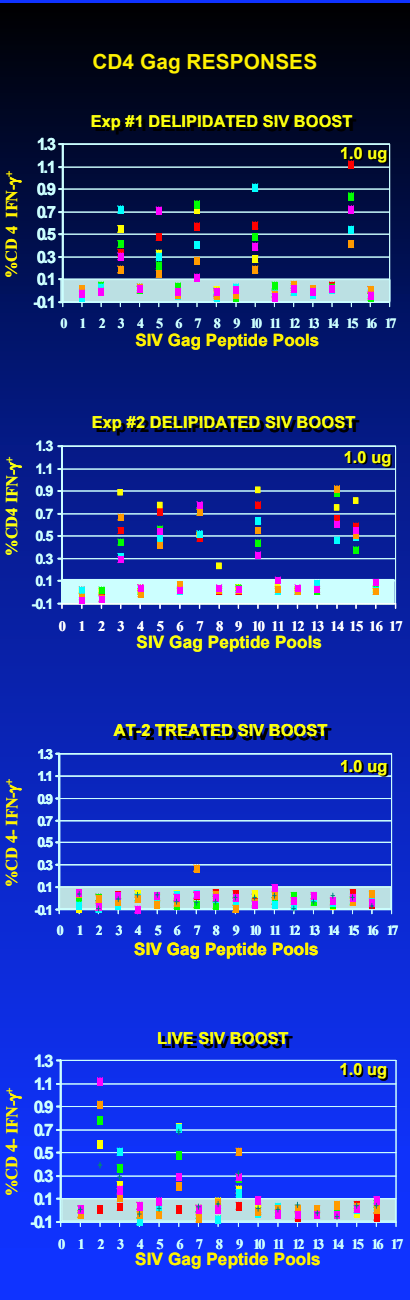
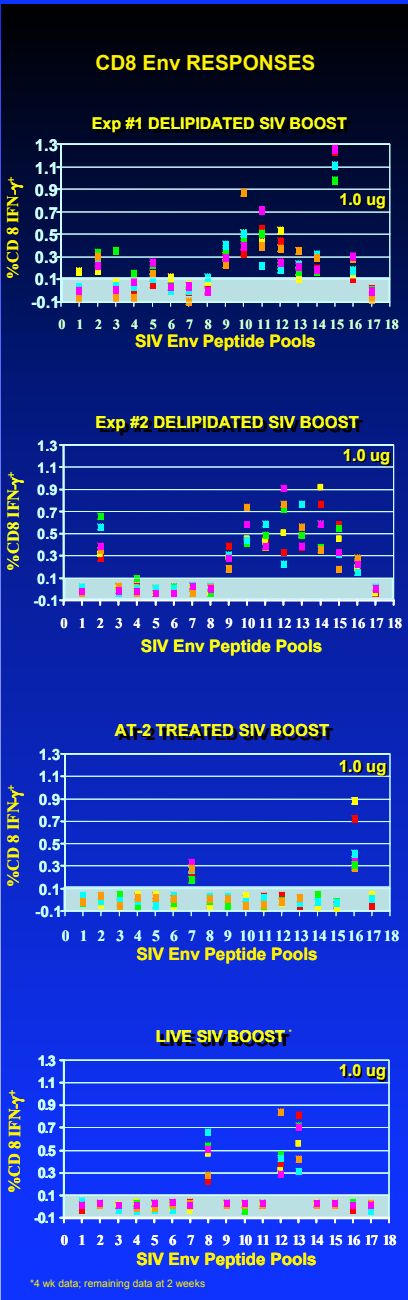
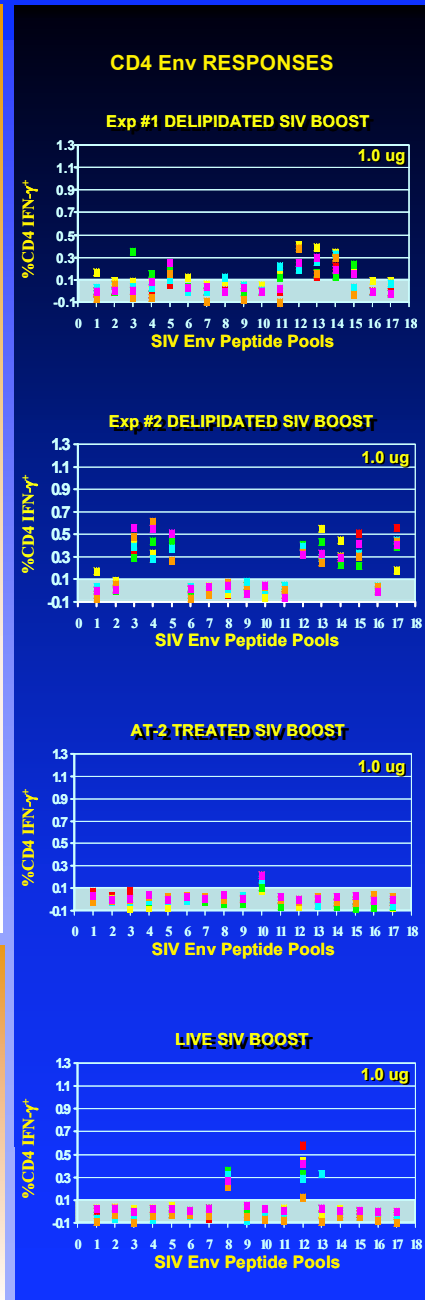
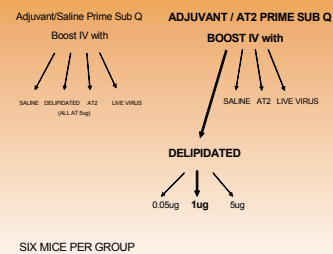
Methods: As proof of principle, Balb/c mice each were immunized with chemically (AT-2)¹ treated SIV-mac251 in Freund's incomplete adjuvant, subQ. Two-four weeks later, groups of such Balb/c mice (n=6/group) were boosted IV with either saline, varied dosages of AT-2 treated SIV-mac251, live or delipidated SIV-mac251. Controls consisted of non-immunized mice and mice administered the booster dose alone. Spleen cells were obtained and cultured *in vitro* overnight with individual pools of overlapping SIV Env and SIV Gag peptides spanning the entire sequence of SIV Env and Gag. Such spleen cell cultures were then assessed for the ability of CD4⁺ and CD8⁺ T cells to synthesize IFN-gamma by standard intracellular cytokine staining (ICC) and flow cytometry.

Results: Results indicate that delipidated SIV-mac251 led to marked augmentation of the SIV specific cellular responses, with a markedly broader and expanded breadth of SIV Env and SIV Gag peptide specific CD4⁺ and CD8⁺ T cell responses versus AT-2 treated and live virus controls as measured by IFN-gamma synthesis. Duration of response to the exposed epitopes and their therapeutic efficacy need further investigation.

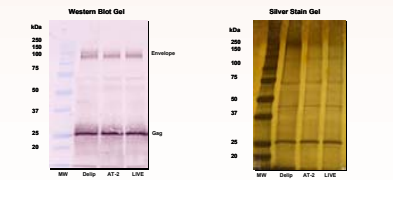
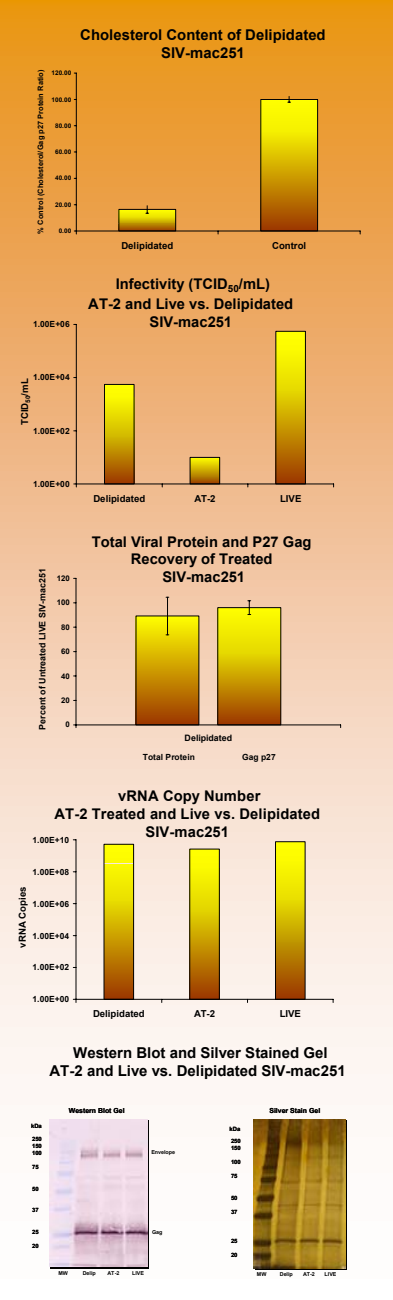
Conclusion: These results, showing a CD4⁺ and CD8⁺ cellular response to a broad array of SIV antigens, exposed by selective removal of the viral lipid coat, hold promise for the development of a novel approach for an autologous therapeutic vaccine for HIV infected patients.

Reference: 1. Rossio, J.L., M.T. Esser, K. Suranarayana, D.K. Schnieder, J.W. Bess, Jr., G.M. Vasquez, T.A. Wiltrout, E. Chertova, M.K. Grimes, Q. Sattentau, L.O. Arthur, L.E. Henderson, and J.D. Lifson. 1998. Inactivation of human immunodeficiency virus type 1 infectivity with preservation of conformational and functional integrity of virion surface proteins. *J Virol* 72:7992.

MOUSE PRIME / BOOST PROTOCOL



¹4 wk data; remaining data at 2 weeks



Results:

*Balb/c mice primed subQ with AT-2 treated virus in Freund's Incomplete Adjuvant and boosted with delipidated SIV-mac251 resulted in unique and broader CD4⁺ and CD8⁺ T cell responses to Env and Gag peptide pools when compared to boosting with live or AT-2-treated SIV-mac251.

*The optimal SIV-mac251 booster dosage was 1ug without adjuvant.

*Repeat experiments (Exp #1 & #2) showed similar results. Amplitude of CD4⁺ responses to Gag peptide pools and amplitude of CD8⁺ responses to both Env and Gag peptides were greater than for CD4⁺ Env peptide pools.

*Experimental controls primed with adjuvant plus saline followed by boosting 2 or 4 weeks later with either saline, live, AT-2 treated or delipidated SIV-mac251, as well as non-immunized mice showed no or negligible responses to the peptide pools.

*SIV-mac251, delipidated by Lipid Sciences, Inc. proprietary process, resulted in at least a 2 log reduction in infectivity (TCID₅₀/mL) compared to live virus.

*Silver staining, Western Blot analysis and biochemical analysis of delipidated SIV-mac251 demonstrated conservation of total protein and viral proteins such as Gag and Env.

*Significant depletion of viral cholesterol (>80%) was achieved as a result of the delipidation process when compared to live virus.

Conclusions:

*Delipidation of SIV-mac251 viral membranes (measured by cholesterol depletion) without significant loss of total protein, viral RNA or p27, resulted in a viral preparation capable of eliciting a broader array of CD4⁺ and CD8⁺ T cell responses to overlapping Env and Gag peptide pools in mice than live or AT-2 treated virus in a prime-boost model.

*The amount of total protein needed to elicit such responses was only 1µg without adjuvant.

*The amplitude (number of cells responding) and breadth (number of peptide pools responding) of both the CD4⁺ and CD8⁺ T cell responses were best in mice boosted with delipidated SIV-mac251.

*These data suggest that delipidated SIV is more efficiently presented and processed via both the MHC Class I and MHC Class II pathways than live or AT-2 treated virus, leading to a more robust CD4⁺ and CD8⁺ T cell response.

*This novel approach, utilizing delipidated virus, has potential application in designing more effective vaccines against enveloped viruses such as HIV.

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