

# 8th International Workshop on Adverse Drug Reactions and Lipodystrophy in HIV



San Francisco, California - September 24 - 26, 2006

## LUMINEX CYTOKINE EXPRESSION ARRAY IN SUBCUTANEOUS FAT ADIPOCYTES, PREADIPOCYTES AND MACROPHAGES IN HIV-LIPOATROPHIC PATIENTS

*Antiviral Therapy* 2006; 11:L25 (abstract no. 37)

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**BACKGROUND:** An increase in proinflammatory cytokines has been found in fat tissue of HIV+ lipodystrophic patients. However, the etiology of the origin of this cytokine production has yet to be elucidated. Our hypothesis is that cells in fat tissue, like adipocytes, preadipocytes, and macrophages can be isolated and cultured separately to assess their pro-inflammatory cytokine production.

**METHODS:** Subcutaneous (SC) adipose tissue biopsies were obtained from HIV+ lipodystrophic and HIV-subjects for flow cytometry analysis ( $n=5$ ) and Luminex cytokine array assessment ( $n=6$ ). Tissue weight excised ranged from 350–950 mg. Adipocytes and preadipocyte pellets (including macrophages) were isolated from the SC tissue using collagenase and centrifugation. Preadipocyte fractions were analysed by flow cytometer for CD68+ (macrophage) cells. Adipocytes and the preadipocyte (+ macrophage) cells were cultured and supernatants were obtained at 24 hours. Cytokine/chemokine levels were then assessed using the Luminex multi-cytokine panel protein array for: Eotaxin, GM-CSF, IFN $\gamma$ , IL-10, IL-12(p40), IL-12(p70), IL-13, IL-15, IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IP-10, MCP-1, MIP-1, RANTES, and TNF $\alpha$ .

**RESULTS:** There is a 50-70% higher level of CD68+ cells in HIV+ ( $n=3$ ) adipose tissue than HIV- ( $n=2$ ), as shown by flow cytometry. The cytokine panels for HIV+lipodystrophic versus HIV negative indicated that out of 22 cytokine/chemokines: 17 recorded above background for preadipocytes: Eotaxin, GM-CSF, INF $\gamma$ , IL-10, IL12(p40), IL-12(p70), IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-6, IL-8, Rantes, IL-15, IP-10, MCP-1, MIP-1, TNF- $\alpha$ ), and 12 for adipocytes: Eotaxin, GM-CSF, INF $\gamma$ , IL-10, IL12(p40), IL-12(p70), IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-6, IL-8, Rantes). The cytokine/chemokine pg/ml indicated a trend for HIV +lipodystrophic versus HIV negative showing differential expression in both adipocytes and the

preadipocyte (+ macrophage) cells. In HIV+lipoatrophic there was a 100% decrease in IL-1 $\alpha$ , IL-6, and IL-8 and a 100% increase in IL-1 $\beta$ , in both adipocytes and the preadipocyte (+ macrophage) fraction, compared to HIV- individuals.

**CONCLUSIONS:** This technology will facilitate further analyses of cytokine expression in different cell components of fat tissue.

**Acknowledgements:** This research was supported by NIH, USA (HL085025, AI068525, AI060409, and RR16467).

2006-09-24

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