Interferon-alpha Treated Pre-adipocyte Fibroblasts Selectively Express Type I Interferon Gene Signature in Both Control and Diet-induced Obesity Models

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Introduction & Background

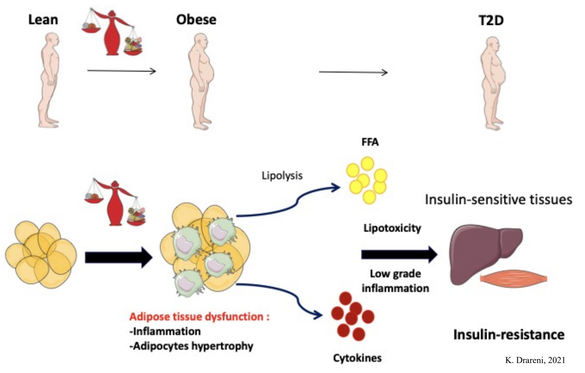

Figure 1. Obesity is associated with adipocyte inflammation and hypertrophy, giving rise to lipotoxicity, chronic low-grade inflammation, and pathogenesis of metabolic syndrome. Due to its various associated co-morbidities, obesity remains a rapidly growing health threat worldwide, driven by a high fat diet (HFD). Therefore, it is crucial to further elucidate the impact of HFD on adipose tissue (AT) expansion, adipogenesis, and pathogenesis of metabolic syndrome. HFD has been shown to drive inflammation and metabolic dysfunction through type I interferon (IFN) signaling, a viral response pathway in which IFN-α and IFN-β cytokines bind receptors IFNAR1 and IFNAR2 to induce its gene signature.[1] Several studies have also demonstrated that obesity-associated type I IFN signaling can play a role in inducing insulin resistance, mitigating the ability of cells to respond to insulin-mediated glucose from the bloodstream into tissues.[2, 3] Moreover, obesity has been linked to an increase in the number of immune cells in AT that secrete cytokines involved in chronic low-grade inflammation that is characteristic of obesity-associated pathophysiology, namely type II diabetes.[4] A hallmark cytokine is IFN, which provides innate resistance against a broad range of viral infections: Type I IFNs predominately involve IFN-α and IFN-β; type II IFNs consist of only IFN-γ. Following the binding of type I IFN to IFNAR, JAK-mediated phosphorylation activates a STAT complex in the plasma membrane, inducing nuclear translocation and distinct gene programs:

- ISGF3 complex (STAT1, STAT2, and IRF9) binds ISRE sequences→ activate antiviral genes OAS and MX1; STAT1 homodimers bind GAS→ induce pro-inflammatory genes IRF1 and CXCL9
- STAT3 homodimers induce transcriptional repressors→ indirectly suppress pro-inflammatory gene expression
- STAT3 bound by SIN3A→ promotes de-acetylation of STAT3 and histones→ suppresses induction of direct STAT3 target genes[5]

While the mechanism-of-action has yet to be fully elucidated, HFD-driven type I IFN signaling presents a potential target in treating a broad spectrum of obesity-associated co-morbidities.

Study Rationale & Aims

- Treat primary stromovascular fraction cells (SVFs) and 3T3L1 fibroblast cell line with IFN-α and IFN-γ to recapitulate phenotype of PDGRF-α+ BST2+ cell cluster
- Reveal contribution of PDGRF-α+ BST2+ cell cluster in driving inflammation implicated in obesity-associated disease pathogenesis
- Elucidate capacity of type 1 IFN to regulate inflammatory features in adipocytes and modulate process of adipogenesis
- Culture 3T3L1 fibroblasts with medium of IFN-treated SVFs to determine whether SVFs secrete soluble factors that mitigate ability of neighboring cells to differentiate into adipocytes
- Determine whether type 1 IFN signaling plays a role in the dysfunction of fibroblasts that drives obesity-associated metabolic syndrome

Preliminary Data

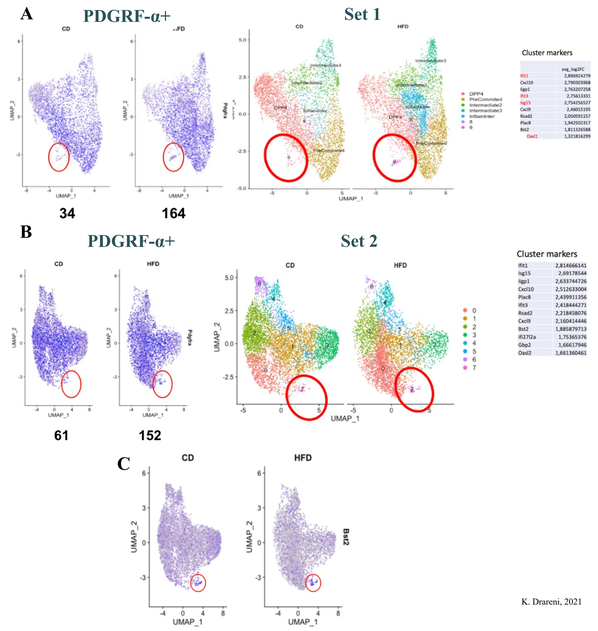

Figure 2. UMAPS generated via single-cell RNA sequencing on fibroblasts isolated from epididymal white AT (EWAT) of HFD vs. control diet (CD) mice. Fibroblasts were sub-setted from EWAT of 22-week-old C57BL/6J mice fed HFD for 16 weeks. CD mice under same conditions were designated controls. Analysis was run on cluster marker genes for type I IFN signature.

A. Set 1: Increased cluster for type I IFN signature and larger fibroblast population in HFD (164) compared to CD (34). Type I IFN-specific signature genes Isg15 and Ifit3 both have relative fold change levels of 2.8.
B. Set 2: Increased cluster for type I IFN signature and larger fibroblast population in HFD (152) compared to CD (61). Type I IFN-specific signature genes Isg15 and Ifit3 have relative fold change levels of 2.7 and 2.4, respectively.
C. Cell sorting EWAT cell population of HFD vs. CD. HFD demonstrated increased cluster for BST2, a specific marker for type I IFN-producing cells in naïve mice

Methods

1. Cell Culture: EWAT harvested from 12-week C57BL/6 CD mice. 3T3L1s were seeded at passage 3. Fibroblasts were isolated and treated with IFN-α-1A at 0, 10, 100, 1000 U/mL concentration; IFN-γ at 0, 2.5, 5, 10 ng/ml concentrations for 24 hours. DMEM F12 was replaced and collected as conditioned media (CM) after 24 hours; SVFs and 3T3L1s underwent RNA isolation and RT-qPCR for type I IFN signature genes.
2. CM: 3T3L1s were seeded at passage 3, treated twice with 2X induction media (-rosiglitazone, -T3) diluted 1:1 with CM from SVFs every 48 hours, then with insulin media for 48 hours. 3T3L1s underwent RNA isolation and RT-qPCR for type I IFN signature genes.
3. Cell sorting EWAT of 10 16-week C57BL/6 mice fed HFD for 10 weeks:
    1. CD45(-): exclude immune cells
    2. CD31(-): exclude endothelial cells
    3. PDGRF-α(+): select for fibroblasts
    4. BST2(+): select for type I IFN population

Results

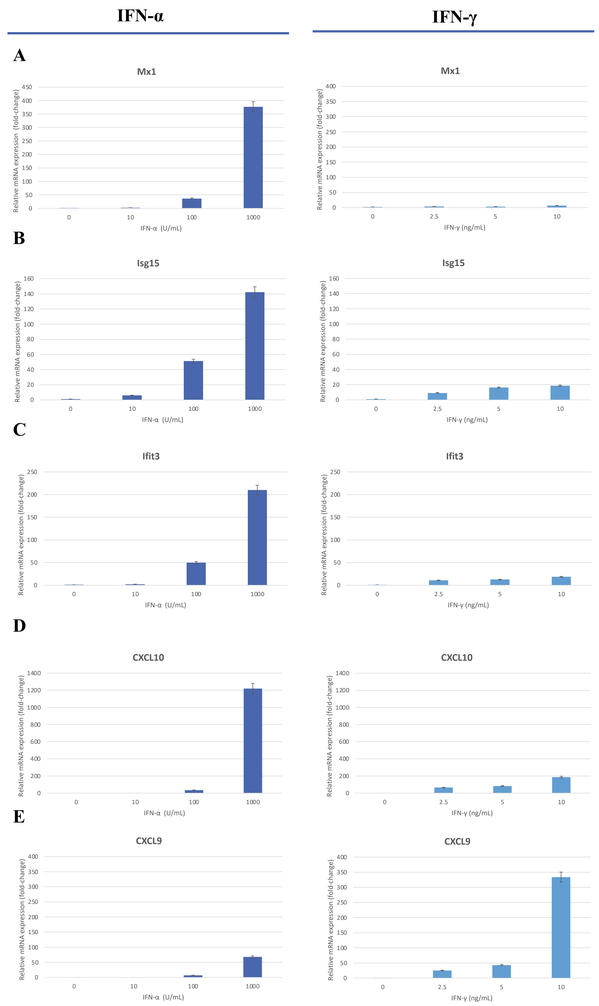

Figure 3. 3T3L1 murine fibroblast cell line expresses marked IFN gene signature with selective response to IFN treatments. 3T3L1s seeded at passage 3 were treated with either IFN-α and IFN-γ at concentrations from respective titration curves as outlined in Methods. RNA was isolated and RT-qPCR was run using IFN signature genes and 18S endogenous control. Delta-delta CT analysis was conducted to determine mRNA expression fold-change relative to negative controls (0 U/mL IFN-α; 0 ng/mL IFN-γ concentrations). Mx1, Isg15, Ifit3 are specific to type I IFN gene signature; CXCL10 and CXCL9 are induced by both type I and II IFNs.

A. Mx1 expression increased 377-fold from 1000 U/mL IFN-α treatment; substantially lower expression from IFN-γ treatment at all titration points
B. Isg15 expression increased 142-fold from 1000 U/mL IFN-α treatment; substantially lower expression from IFN-γ treatment at all titration points
C. Ifit3 expression increased 210-fold from 1000 U/mL IFN-α treatment; substantially lower expression from IFN-γ treatment at all titration points
D. CXCL10 expression increased 1219-fold from 1000 U/mL IFN-α treatment; substantially lower expression from IFN-γ treatment at all titration points
E. CXCL9 expression increased 334-fold from 1000 U/mL IFN-α treatment; substantially lower expression from IFN-γ treatment at all titration points

Conclusions & Future Directions

Upon viral infection, the type I IFN response is rapidly induced, playing a central role in activating the innate immune response and priming the adaptive immune system. Of note,. numerous cell types including lymphocytes, macrophages, dendritic cells (DCs), plasmacytoid DCs, and fibroblasts secrete type I IFNs, and type I IFNAR is ubiquitously expressed in cells throughout the body.[6] Taken together, this implies that many tissues possess the ability to respond to type I IFN cytokines, ultimately giving rise to a state of chronic low-grade inflammation. In this study, 3T3L1s were treated with varying concentrations of IFN-α and IFN-γ; mRNA expression levels of IFN signature genes were examined. From qPCR analyses, it was clearly determined that IFN-α treatment of 1000 U/mL had induced the strongest type I IFN signature response, and IFN-α treatment increased the expression of type I IFN-specific genes Mx1, Isg15, and Ifit3 by several-hundred-fold compared to negative controls. These fold changes were significantly greater than expression levels from IFN-g treatment, suggesting that IFN-α cytokines selectively bind IFNAR receptors on fibroblasts to induce type I IFN responses. Mx1 participates in the cellular antiviral response by encoding a protein that antagonizes replication of RNA and DNA viruses.[7] Isg15 similarly inhibits viral replication in a conjugation-dependent manner by functioning as a bona-fide antiviral protein.[8] Moreover, Ifit3 has been shown to modulate apoptosis and cell proliferation[9], generating a pro-inflammatory environment within AT. These experimental results run parallel with the finding that ectopic type I IFN signaling promotes insulin resistance in adipocytes[10], in turn contributing to the dysregulation of glucose homeostasis and inflammation-associated metabolic syndrome. Future studies should consider analyzing the effects of blocking type I IFN signaling in AT, which can play a crucial role in ameliorating diet- and obesity-associated insulin resistance.

While IFN-treated 3T3L1s demonstrated both the most pronounced expression levels and distinct differences in expression levels between type I and type II IFN treatments, similar patterns were observed in IFN-treated EWAT SVFs as well as in 3T3L1s treated with CM 2X induction cocktail. Further investigations will involve treating SVFs and 3T3L1s with higher concentrations of IFN-α to optimize treatment conditions. Experiments will be replicated in 22-week-old C57BL/6J mice fed HFD for 16 weeks, the same conditions used in the cell sorting experiment. ELISA will be conducted on CM from IFN-treated EWAT SVFs and 3T3L1s to quantify levels of soluble proteins released. Finally, the cell sorting experiment will be repeated with 15 mice to yield a higher number of sorted PDGRF-α+ BST2+ fibroblasts, to be plated and analyzed via RT-qPCR; remaining EWAT SVFs will be designated as negative control.

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