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Effects of HIV Proteins on Macrophage Response to MAI

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Effects of HIV proteins on macrophage response to MAI.

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Background

NTM: Nontuberculous Mycobacteria
- *Mycobacterium avium* (MAV) is one of the primary NTM involved in pathological pulmonary infections, especially in immunocompromised patients including those with cystic fibrosis, chronic obstructive pulmonary disease, and HIV/AIDS
- Of concern is the high level of antimicrobial resistance displayed by these organisms, which complicates treatment and potential successful outcomes.

HIV:
- Infection by HIV remains a major global threat with ~34 million individuals living with HIV worldwide. Among these individuals, one-third are co-infected with mycobacteria (TB) and Non-Tuberculous Mycobacteria (NTM)
- HIV infection and disease progression damages host immune cell function resulting in an immunocompromised state with patients more susceptible to opportunistic pulmonary infections

Macrophage:
- Macrophages are the primary host cells that initiate an immune response to NTM
- Alveolar and lung tissue macrophages are the first line of defense against NTM infections, initiating the innate immune response during the initial infection, involving identification of bacterial pattern recognition receptors (PRR), phagocytosis, and subsequent lysosomal degradation

Mitochondria:
- PGC-1α is the master transcriptional regulator of mitochondrial biogenesis
- Mitochondrial biogenesis is needed to generate new mitochondria to optimize ATP production which is needed for key cellular functions such as phagocytosis and bacterial killing in macrophages.
- Efficient bacterial killing requires a high rate of metabolic activity, making macrophage mitochondrial activity a key component in bacterial clearance and immune cell function.

Experimental Design

Hypothesis: Treatment of macrophages with HIV proteins induces mitochondrial dysfunction mediated by reduced PGC-1α expression resulting in impaired immune response to MAI.

Objective:
- Determine the mechanism by which HIV proteins impairs macrophage function – we hypothesize that this is due to attenuated mitochondrial biogenesis from reduced PGC-1α expression.

Methods

Methods:
- Cultured RAW 246.7 macrophages and mycobacterium avium intracellular (MAI) and administered the cells with HIV proteins: gp120, tat [100ng/mL], 24-hour pretreatment of HIV proteins prior to infection
- Analyzed protein expression PGC-1α through western blotting and immunocytochemistry
- Examined mRNA expression of PGC-1α through qRT-PCR
- Investigated mitochondrial membrane integrity with Mito Tracker and TMRM

Expression of PGC1 alpha and TFAM is attenuated by HIV viral proteins

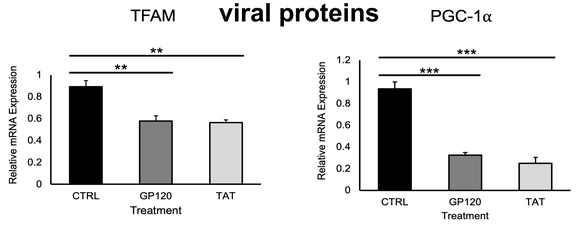

Figure 1: Relative mRNA expression of TFAM decreases when the cell is treated with GP120 and TAT as well as the even more dramatic decrease in relative mRNA expression of PGC-1**α** in GP120 and TAT treated cells suggesting dysfunction in mitochondrial functions such as replication.

Mitochondrial membrane potential is attenuated in macrophages treated with HIV viral proteins and non-TB mycobacteria

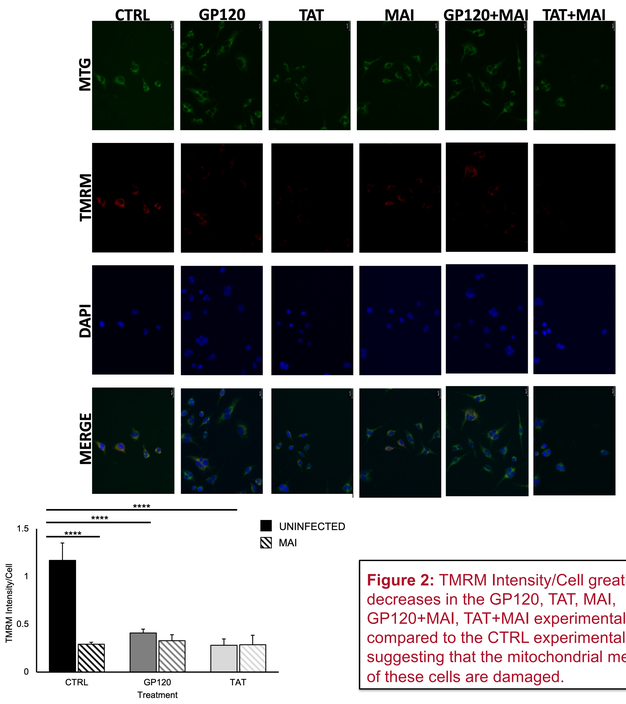

Figure 2: TMRM Intensity/Cell greatly decreases in the GP120, TAT, MAI, GP120+MAI, TAT+MAI experimental groups compared to the CTRL experimental group suggesting that the mitochondrial membrane of these cells are damaged.

Expression of PGC1 alpha and TFAM proteins is attenuated in macrophages treated with HIV viral proteins and infected with NTM

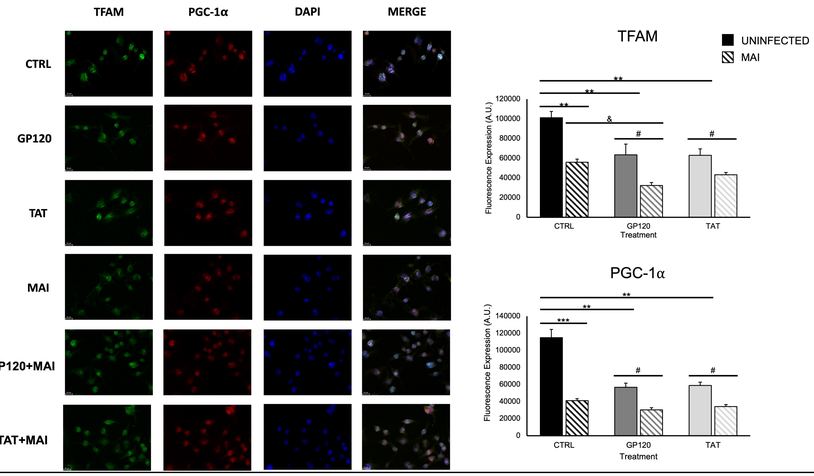

Figure 3: Decreasing expression of TFAM and PGC-1**α** in cells treated with GP120 and TAT as well as the further decrease in these protein expressions when the cells are infected with MAI suggesting that MAI is more harmful to protein expression than treatment with GP120 or TAT only and treatment with GP120 or TAT along with infection of MAI is the most devastating to protein expression.

Conclusion and Future Directions

Conclusions:
- HIV proteins, GP120 and TAT, along with MAI damage the mitochondrial membrane decreasing the cell's ability to perform important cellular tasks and ultimately results in cell death.
- HIV proteins, GP120 and TAT, and MAI decrease the expression of TFAM and PGC-1**α** proteins resulting in impairment of mitochondrial biogenesis making the cell more suspectable to disease and total dysfunction.

Future Directions:
- Investigate the impact of restoring mitochondrial biogenesis by activating PGC-1α – we hypothesize that activation of PGC-1α will enhance mitochondrial biogenesis with enhanced macrophage phagocytosis and bacterial killing.
- Investigate the cellular pathways impaired by GP120 and TAT.

References

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- Maurice NM, Bedi B, Yuan Z, Goldberg JB, Koval M, Hart CM, Sadikot RT. Pseudomonas aeruginosa Induced Host Epithelial Cell Mitochondrial Dysfunction. Sci Rep. 2019 Aug 15;9(1):11929.