HIV-Mediated Activation of the NLRP3-Inflammasome

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Abstract

The focus of this project is to elucidate a key mechanism of the host response to HIV infection. Patients plagued by long-term HIV infection develop chronic ageing-related inflammatory conditions earlier in life than is normally expected. These inflammatory conditions and chronic immune activation have been linked to the action of a protein in the NLR inflammasome family, specifically NLRP3, which recognizes intracellular pathogen molecules and promotes inflammatory responses aimed at controlling the pathogen. Inflammation induced by NLRP3 can be suppressed with docosahexaenoic acid (DHA), which is therefore a potential treatment option [10].

Introduction

We hypothesize that activation of the NLRP3-inflammasome by HIV-1 components can be suppressed by treatment with DHA.

Materials & Methods

Cell culture, DNA transfection, and inflammasome reconstitution. Human monocytic cell line THP-1 (differentiated with PMA) and the epithelial cell line A2327 were cultured in RPMI 1640 or DMEM medium, respectively, with 10% FBS, 1% glutamine and 0.1% Penicillin/Streptomycin. DNA transfection was performed using FugeneHD with 3:1 ratio of FugeneHD:DNA. For inflammasome reconstitution, A2327 cells were seeded in 24-well plates and following transfection, cells were transfected with plasmids encoding pro-caspase-1 (30μg), pro-IL-1β (200ng) and ASC (80μg) with or without NLRP3 (100ng) and with or without HIV DNA (200ng). After 24 hrs, transfection supernatants were collected by centrifugation and secreted IL-1β was measured by ELISA (mg/mL) as per the manufacturer's instructions. THP-1 cells were infected with UV-irradiated HIV-1 at an MOI of 10.

Results

Activation of the NLRP3 inflammasome is increased by HIV-1 replication. Human monocytic cell line THP-1 was differentiated for 24 hrs with PMA (20ng/mL). DNA transfection was performed to introduce an HIV-1 Env-coding plasmid and transfected cells were infected with HIV-1 (MOI 0.1). Secreted IL-1β level was measured in supernatants by ELISA (ng/mL).

Future Directions

The NLRP3-inflammasome is activated by genomic HIV-1 CDNA (Figure 3)

The NLRP3-inflammasome solely contributed to the increased IL-1β secretion seen in the experiment from figure 3. (Figure 4) - endogenously expressed inflammasome proteins showed decreased expression

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References