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EXPRESSION OF ACUTE-PHASE PROTEINS BY ELAD® C3A CELLS

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BACKGROUND

A key pathogenesis of alcohol-induced liver decompensation (AILD) involves unregulated systemic inflammation[1]. AILD patients have elevated plasma levels of inflammatory factors such as IL-1β, IL-6, lipopolysaccharide (LPS), and TNF-α[2-5]. The diseased liver is unable to respond appropriately to these inflammatory mediators. This impaired immune response leaves the patient susceptible to systemic inflammatory syndrome which is associated with increased patient mortality[6].

In the healthy liver, hepatocytes are the major sources of acute-phase proteins (APPs) which contribute to control of systemic inflammation[7]. Healthy hepatocytes respond to IL-1 and IL-6 (leading regulators of the APP response) by producing mediators of inflammatory resolution such as IL-1 receptor antagonist (IL-1Ra) and α1-antitrypsin (ATT)[4,8,9]. IL-1Ra and ATT have a demonstrated ability to mitigate systemic inflammation through competitive inhibition, protease inhibition, and blocking production of inflammatory signaling cascades[9,10].

Providing anti-inflammatory AAPs and other immune modulators (e.g. IL-10) to the AILD patient could provide therapeutic benefit.

Therapies, such as anti-TNF-α or steroid dosing have not demonstrated long-term clinical benefit[1,11]. A multi-factor cell-based strategy such as Vital Therapies' (VTL) ELAD® System (an investigational human, cell-based bio-artificial liver support system comprised of four metabolically active ELAD cartridges with ancillary device components and support circuitry intended to continuously treat subjects with acute liver failure for up to 10 days) may be beneficial. The ELAD System is under clinical investigation for several acute forms of liver failure.

OBJECTIVES

The purpose of this study was to evaluate the ability of VTL C3A cells to respond to selected inflammatory mediators (alone or in combination) commonly found in AILD patient plasma by secreting anti-inflammatory factors associated with the resolution of inflammation.

MATERIALS & METHODS

VTL C3A cells were plated in monolayer and incubated with LPS or with inflammatory cytokines (IL-6, IL-1β, and/or TNF-α) for 24, 48, or 54 h. Cytokines were dosed individually or in combination at 0, 1, 10, or 100 ng/mL as indicated in each figure. Separate VTL C3A cells were incubated with LPS at 0, 0.01, 0.1, 1, or 10 EU/mL for 24 h.

Additionally, intact ELAD C3A tissue (VTL C3A cells cultured three-dimensionally between polysulfone hollow fibers) was excised from ELAD C3A cell cartridges and dosed in conjunction with 10 ng/mL IL-1β and 10 ng/mL IL-6 for 24 h. The supernatants from these monolayer VTL C3A cell and ELAD C3A tissue experiments were assayed for IL-1Ra, AAT, IL-10, or albumin via in-house and commercial ELISA kits (R&D Systems, abcam), contracted services for multiplex ELISAs (Myriad), or chemiluminescent multiplex assay kits (Aushon).

RESULTS

When monolayer VTL C3A cells were co-incubated with both IL-1β and IL-6, a synergistic response was observed towards increased IL-1Ra secretion, which was further increased at 54 h (Fig 1). Albumin levels were decreased in response to IL-1β and IL-6 in these same monolayer samples at 54 h (Fig 3). However, when monolayer VTL C3A cells were incubated with IL-1β or IL-6 only, secretion of IL-1Ra did not increase above the level of the untreated controls.

AAT, in general, was secreted at concentrations nearly 1,000-fold higher than IL-1Ra in monolayer VTL C3A cells. However, there was no apparent effect on AAT secretion when exposed to either IL-6, IL-1β, or their combination (Fig 2). There was a time-dependent increase in AAT concentrations under all treatment conditions.

Fibrinogen was observed to increase predominantly in IL-6-treated monolayer VTL C3A cultures. Expression was abrogated by addition of IL-1β (Fig 4). α-2 macroglobulin also increased in response to IL-6 but not in response to IL-1 β (Fig 5).

When monolayer VTL C3A cells were incubated with 10 ng/mL IL-1β only, 10 ng/mL IL-6 only, or in combination, the secretion of IL-10 increased above the controls, and further increased at 48 hr. The dosing of IL-6 had a greater effect on IL-10 secretion (Fig 6).

An increase in IL-1Ra (Fig 7), but not in AAT (Fig 8), was also observed when ELAD C3A tissue was incubated with IL-1β and IL-6 (10 ng/mL each) for 24 h. Sample sizes were increased to 6 replicates to help control for variability due to an inability to normalize results to cell counts after dosing.

TNF-α secretion by monolayer VTL C3A cells increased in response to IL-1β and IL-6 in combination (Fig 9). A similar increase was seen in ELAD C3A tissue (data not shown).

TNF-α (1, 10, or 100 ng/mL) alone did not increase secretion of IL-1Ra by monolayer C3A cells, although there was an observed decrease in secretion of IL-1Ra at the highest dose (100 ng/mL) (Fig 10). IL-1β (10 ng/mL) alone and combination with and TNF-α (10 ng/mL each), increased secretion of IL-1Ra (Fig 10). Once again, there was no effect on AAT secretion in any dose group (data not shown).

Direct exposure of monolayer VTL C3A cells to LPS increased IL-1Ra secretion approximately 2-fold at all concentrations evaluated (0.01, 0.1, 1, and 10 EU/mL) (Fig 11). AAT levels were increased only at higher concentrations (1 and 10 EU/mL) (Fig 12).

C-reactive protein was below the lower level of quantitation of the assay (0.012 ng/mL). Haptoglobin levels did not change significantly with any treatment (data not shown).

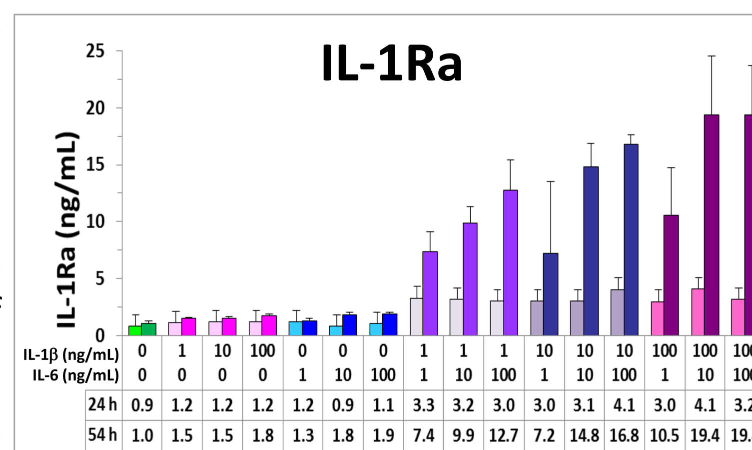

Fig 1. Monolayer C3A cell ($1.3x10^5$ cells/cm^2) secretion of IL-1Ra is upregulated in the combined presence of IL-6 and IL-1β and increases with exposure time. Results are mean ± SD, n=3 biological replicates (left-24 h, right-54 h for each pair of bars).

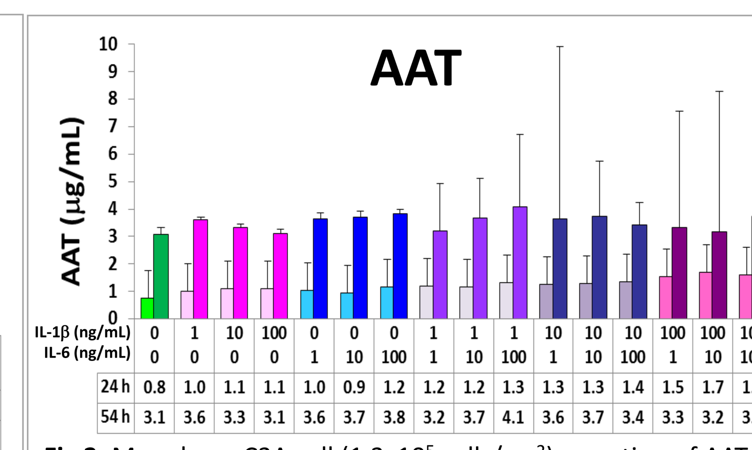

Fig 2. Monolayer C3A cell ($1.3x10^5$ cells/cm^2) secretion of AAT increases with time, but is not affected by exposure to IL-6 or IL-1β individually or in combination. Results are mean ± SD, n=3 biological replicates (left-24 h, right-54 h for each pair of bars).

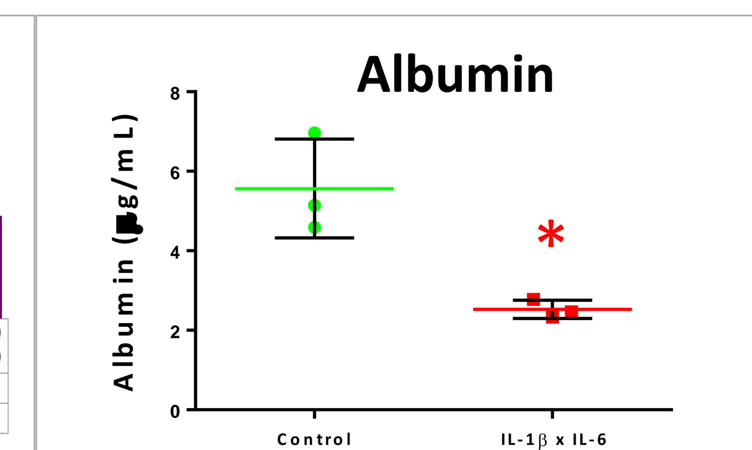

Fig 3. Monolayer C3A cell ($1.3x10^5$ cells/cm^2) secretion of albumin is downregulated at 54 h in the combined presence of IL-6 (10 ng/mL) and IL-1β (10 ng/mL) (*p=0.047), as expected for this APP. Results are mean ± SD, n=3 replicates.

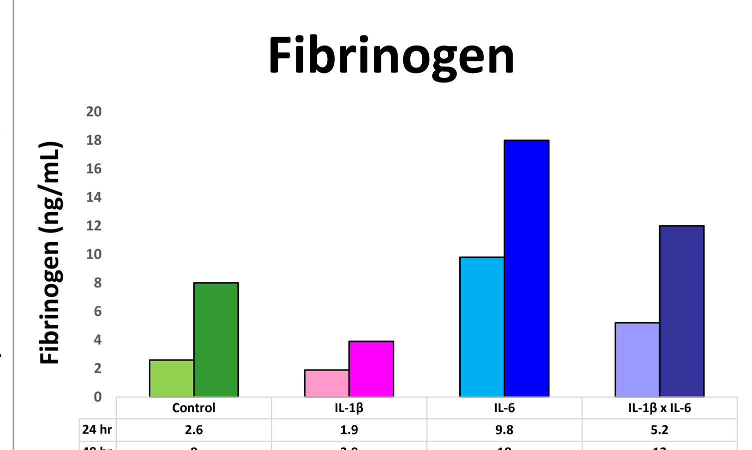

Fig 4. Monolayer C3A cell ($1.3x10^5$ cells/cm^2) secretion of fibrinogen was increased by IL-6 and abrogated by IL-1β. Results are single replicates of pooled triplicate wells.

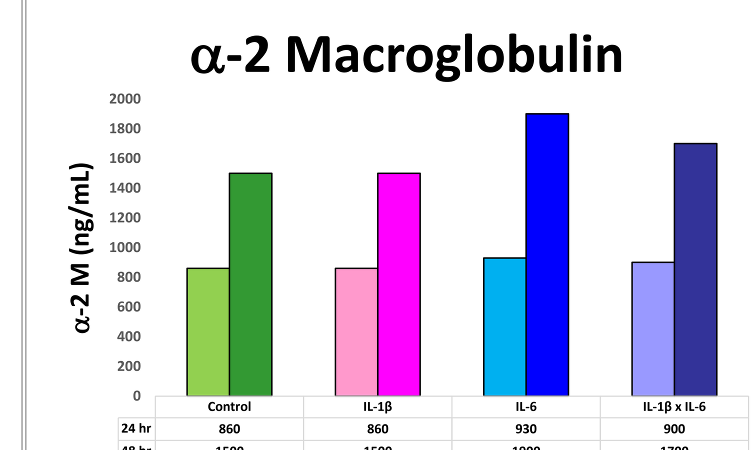

Fig 5. Monolayer C3A cell ($1.3x10^5$ cells/cm^2) secretion of α-2 Macroglobulin (α-2M) appeared modestly upregulated by IL-6 alone. Results are single replicates of pooled triplicate wells.

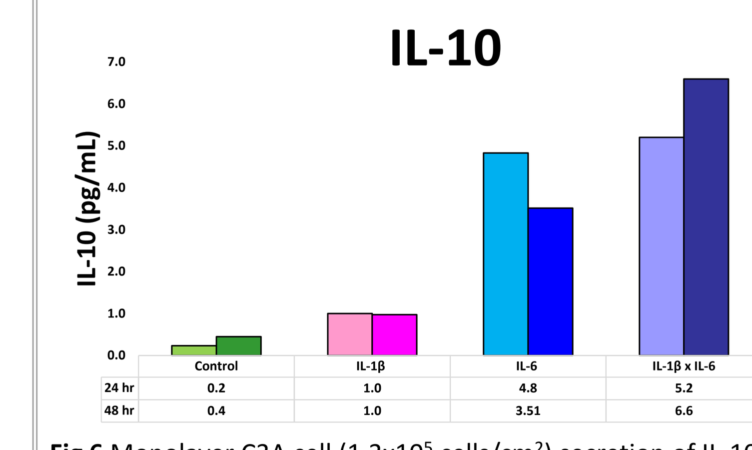

Fig 6 Monolayer C3A cell ($1.3x10^5$ cells/cm^2) secretion of IL-10 is upregulated in the presence of IL-1β or IL-6, and is more driven by IL-6. Results are single replicates of pooled triplicate wells (left-24 h, right-48 h for each pair of bars).

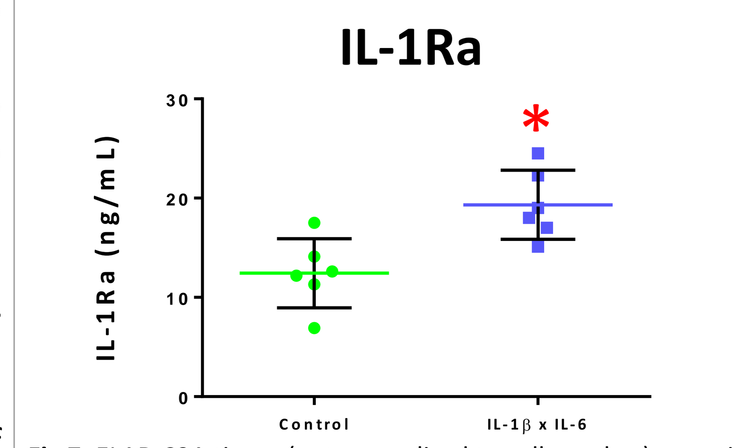

Fig 7. ELAD C3A tissue (not normalized to cell number) secretion of IL-1Ra is also upregulated at 24 h in the combined presence of IL-6 (10 ng/mL) and IL-1β (10 ng/mL) (*p=0.007). Results are mean ± SD, n=6 biological replicates.

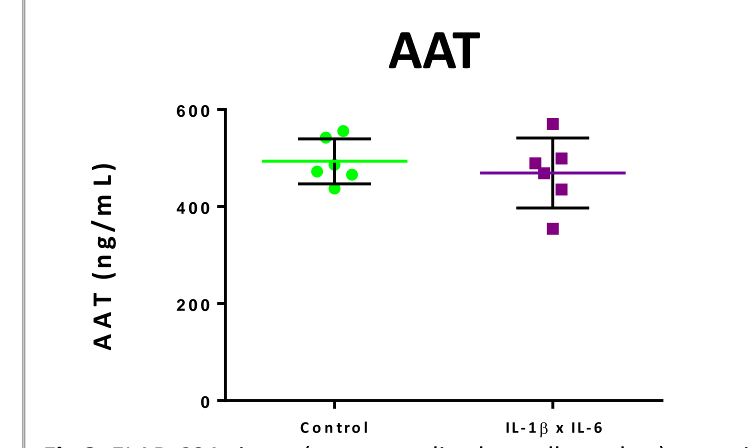

Fig 8. ELAD C3A tissue (not normalized to cell number) secretion of AAT is not upregulated at 24 h in the combined presence of IL-6 (10 ng/mL) and IL-1β (10 ng/mL) (p=0.51). Results are mean ± SD, n=6 biological replicates.

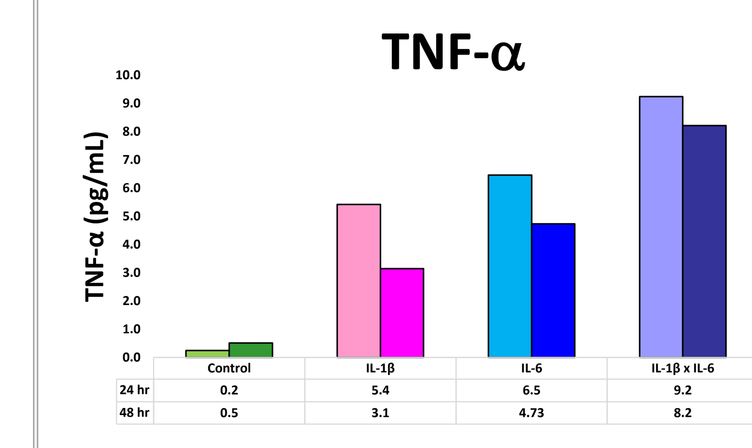

Fig 9. Monolayer C3A cell ($2.6x10^5$ cells/cm^2) secretion of TNFα is upregulated in the presence of IL-1β or IL-6. Results are single replicates of pooled triplicate wells (left-24 h, right-48 h for each pair of bars).

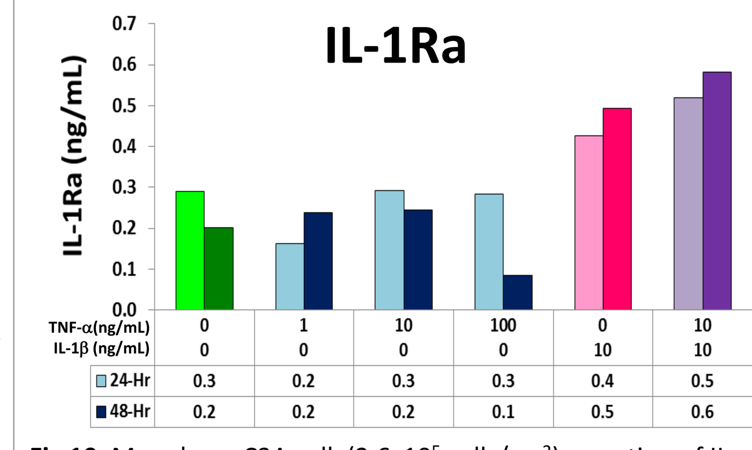

Fig 10. Monolayer C3A cell ($2.6x10^5$ cells/cm^2) secretion of IL-1Ra is upregulated in the presence of IL-1β (10 ng/mL) and combination of IL-1β and TNF-α (10 ng/mL each), but not TNF-α alone. Results are single replicates of pooled triplicate wells.

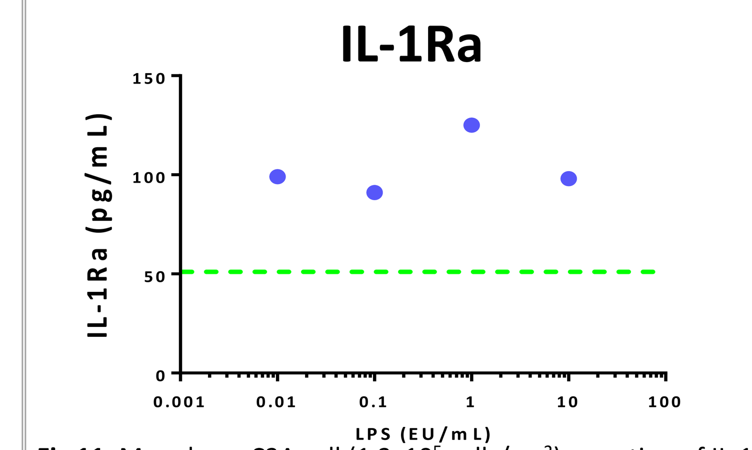

Fig 11. Monolayer C3A cell ($1.3x10^5$ cells/cm^2) secretion of IL-1Ra is upregulated at 24 h in the presence of LPS. Results are single replicates of pooled triplicate wells (green line indicates untreated control response).

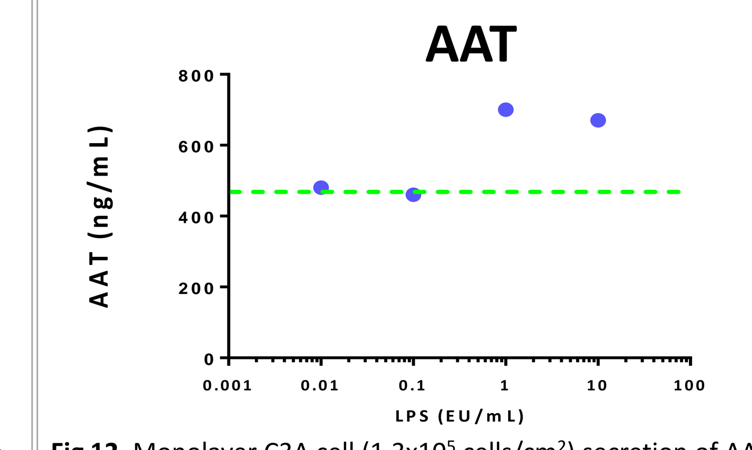

Fig 12. Monolayer C3A cell ($1.3x10^5$ cells/cm^2) secretion of AAT is upregulated at 24 h in the presence of higher concentrations of LPS. Results are single replicates of pooled triplicate wells (green line indicates untreated control response).

DISCUSSION

In these studies, the ability of VTL C3A cells in both monolayer and in ELAD tissue to respond to pro-inflammatory cytokines and key mediators of the acute phase response, IL-1β, IL-6 and TNF-α, and to LPS was demonstrated.

In both monolayer and ELAD tissue, the VTL C3A cells respond to these inflammatory mediators, found elevated in AILD patients, by upregulated and/or constitutive expression of anti-inflammatory APPs.

As is characteristic of an acute phase response, the effects of IL-6 and IL-1β vary dependent upon the resulting factor, and can be inhibitory (e.g. fibrinogen) or enhancing (e.g. IL-1Ra) of each other[7]. Reduced albumin production, in parallel with increased IL-1Ra secretion, is also consistent with an acute phase response.

Exogenous AAT and IL-1Ra have been shown to suppress pro-inflammatory cytokine synthesis by interference with TNF-α and IL-1β pathways and enhancement of IL-10 production[9,10], the latter of which has broad anti-inflammatory properties. It is not clear from these studies whether increased IL-10 production by VTL C3A cells results directly from IL-6 exposure or autocrine effects of IL-1Ra.

VTL C3A cells produced low, yet elevated, levels of TNF-α in response to IL-6, IL-1β and the combination. However TNF-α did not significantly impact APP expression except when dosed 10,000-fold higher than measured in culture.

Reduction of pro-inflammatory cytokines and increases in anti-inflammatory APPs in response to elevated cytokines and LPS in AILD patients may contribute to resolution of inflammation by the ELAD System.

CONCLUSIONS

VTL C3A cells secrete anti-inflammatory factors both constitutively and in response to co-incubation with IL-1β and IL-6. Their response is dynamic, exhibiting temporal and dose-dependent secretion of anti-inflammatory mediators, IL-1Ra and AAT. Additionally, VTL C3A cells upregulate IL-1Ra and AAT in response to LPS.

An inflammation resolution response may represent one of the multiple mechanisms for the therapeutic benefit resolution in AILD patients treated with the ELAD System.

ACKNOWLEDGMENTS

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