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International Symposium on Albumin Dialysis

HEPATIC INFLAMMATION AND CELLULAR THERAPY

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VITAL THERAPIES™

TARGETING LIVER DISEASE

BACKGROUND

Alcoholic hepatitis (AH) patients have unregulated systemic inflammation, elevated levels of pro-inflammatory cytokines and compromised immune cell function, in part due to increased gut permeability from chronic alcohol consumption and translocation of bacteria/endotoxin to the circulation.[1-6] Healthy hepatocytes respond to IL-1 and IL-6 by producing acute-phase reaction mediators of inflammatory resolution such as IL-1Ra and ATT.[4,7-10]

Steroidal therapy and other anti-inflammation treatments have not yet demonstrated clinical benefit.[1,11-12] Cell-based therapies may offer advantages over monotherapies and are the focus of current clinical investigation (e.g. the ELAD System).

OBJECTIVES

The purpose of this study was to evaluate the ability of VTL C3A cells (the viable cellular component of the ELAD System) to respond to selected inflammatory mediators (alone or in combination) commonly found in AH patient plasma by secreting anti-inflammatory factors associated with the resolution of inflammation and to evaluate immune cell function when exposed to VTL C3A cell-secreted factors.

MATERIALS & METHODS

Vital Therapies' (VTL) ELAD® System is an investigational extracorporeal human hepatic cell-based liver treatment comprised of four metabolically-active ELAD cartridges with ancillary device components and support circuitry intended to continuously treat subjects with liver failure secondary to acute hepatocellular insult and alcohol use for up to 5 days.

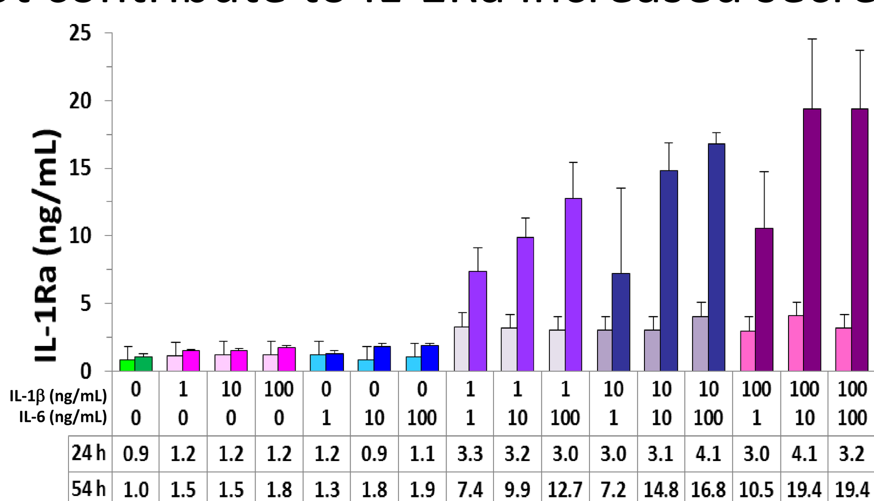
VTL C3A cells were plated in monolayer or as three-dimensional tissue (from ELAD cartridges) and dosed with IL-6, IL-1β, and/or TNFα (0-100 ng/mL) individually or in combination over time. Separate studies dosed VTL C3A cells with LPS (0-10 EU/mL). Cell supernatants were immunoassayed for various acute-phase proteins, cytokines, and other inflammatory mediators.

Peripheral blood neutrophils were isolated from healthy volunteers and exposed to plasma from disease severity-matched control subjects or ELAD-treated subjects to measure oxidative burst and phagocytic capacity. Cytokine levels from subject samples were assayed prior to and 24 h after start of treatment**.**

THP-1 monocytic cells were polarized to pro-inflammatory M1 macrophage phenotype and assayed for cytokine production and phagocytosis in the presence/absence of exposure to VTL C3A-secreted factors.

RESULTS

VTL C3A-secreted levels of IL-1Ra increased in response to IL-6 and IL-1β in a synergistic and time-dependent manner (Fig 1). IL-1Ra levels were not attenuated in the presence of anti-AAT neutralizing antibody (data not shown), suggesting that AAT does not contribute to IL-1Ra increased secretion.

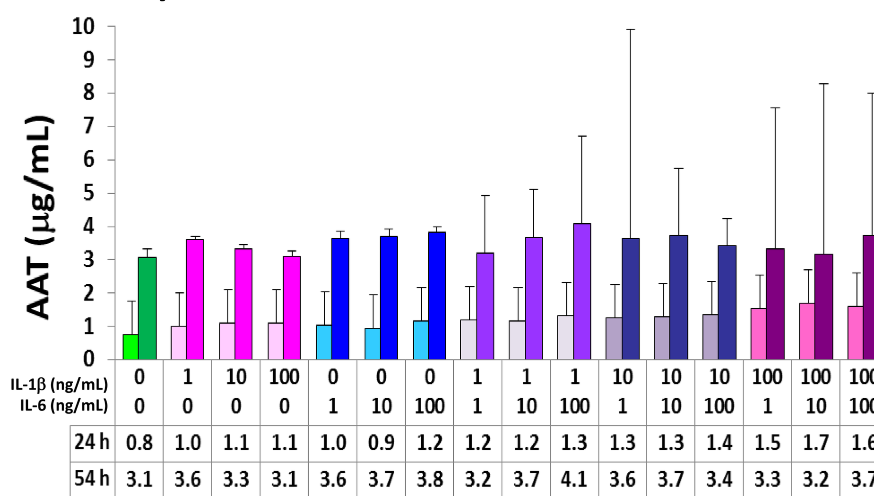


IL-1β (ng/mL)	0	1	10	100	0	0	0	1	1	1	10	10	10	100	100	100
IL-6 (ng/mL)	0	0	0	0	1	10	100	1	10	100	1	10	100	1	10	100
24 h	0.9	1.2	1.2	1.2	1.2	0.9	1.1	3.3	3.2	3.0	3.0	3.1	4.1	3.0	4.1	3.2
54 h	1.0	1.5	1.5	1.8	1.3	1.8	1.9	7.4	9.9	12.7	7.2	14.8	16.8	10.5	19.4	19.4

Fig 1. Monolayer C3A cell (1.3×10^5 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).

RESULTS (cont.)

Mean AAT levels were not affected by exposure to either IL-6, IL-1β, or their combination, and increased only with time.



IL-1β (ng/mL)	0	1	10	100	0	0	0	1	1	1	10	10	10	100	100	100
IL-6 (ng/mL)	0	0	0	0	1	10	100	1	10	100	1	10	100	1	10	100
24 h	0.8	1.0	1.1	1.1	1.0	0.9	1.2	1.2	1.2	1.3	1.3	1.3	1.4	1.5	1.7	1.6
54 h	3.1	3.6	3.3	3.1	3.6	3.7	3.8	3.2	3.7	4.1	3.6	3.7	3.4	3.3	3.2	3.7

Fig 2. Monolayer C3A cell (1.3×10^5 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).

An increase in IL-1Ra (Fig 3), but not in AAT (Fig 4), was also observed when ELAD C3A tissue was incubated with IL-1β and IL-6 (10 ng/mL each) for 24 h.

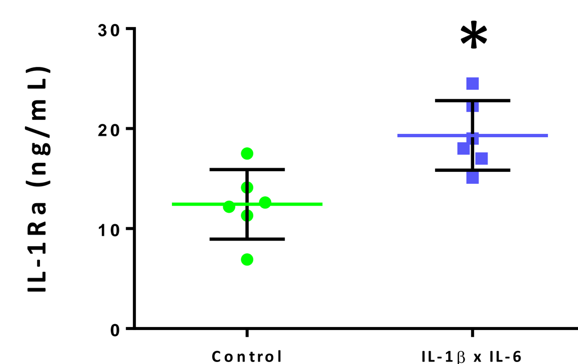


Fig 3. ELAD C3A tissue (not normalized to cell number) secretion of IL-1Ra is also upregulated at 24 h in the combined presence of IL-6 (10 ng/mL) and IL-1β (10 ng/mL) (*p=0.007). Results are mean ± SD, n=6 biological replicates.

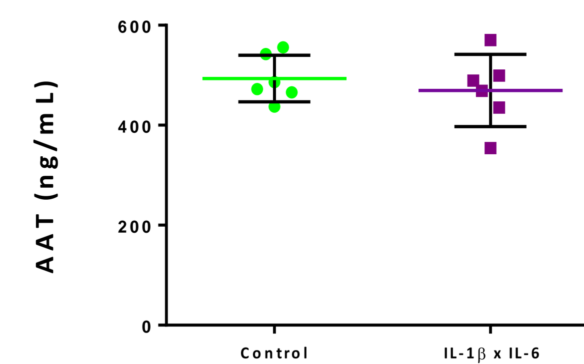


Fig 4. ELAD C3A tissue (not normalized to cell number) secretion of AAT is not upregulated at 24 h in the combined presence of IL-6 (10 ng/mL) and IL-1β (10 ng/mL) (p=0.51). Results are mean ± SD, n=6 biological replicates.

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 5). AAT levels were increased only at higher concentrations (1 and 10 EU/mL) (Fig 6).

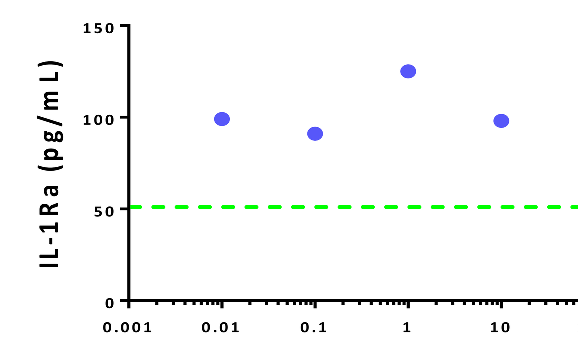


Fig 5. Monolayer C3A cell (1.3×10^5 cells/cm^2) secretion of IL-1Ra is upregulated at 24 h in the presence of LPS. Results are single replicates of pooled triplicate wells (green line indicates untreated control response).

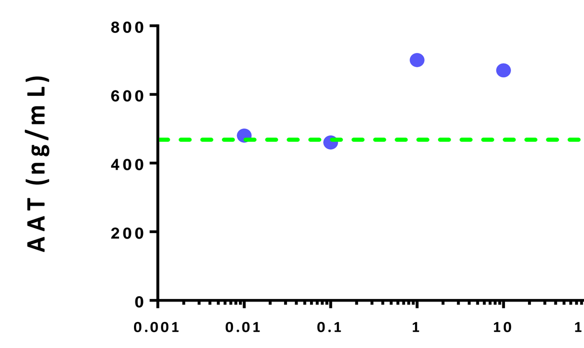


Fig 6. Monolayer C3A cell (1.3×10^5 cells/cm^2) secretion of AAT is upregulated at 24 h in the presence of higher concentrations of LPS. Results are single replicates of pooled triplicate wells (green line indicates untreated control response).

Albumin levels were decreased in response to IL-1β and IL-6 in these same monolayer samples indicating a normal functioning acute-phase response (Fig 7). IL-10 secretion was upregulated in VTL C3A cells greater when exposed to IL-6 than IL-1β (Fig 8).

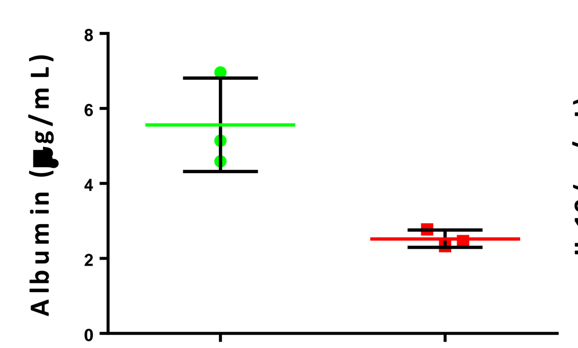
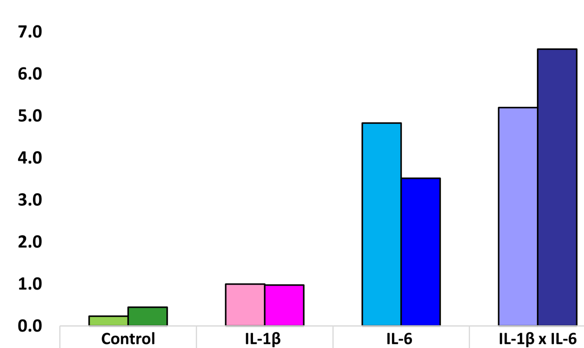


Fig 7. Monolayer C3A cell (1.3×10^5 cells/cm^2) secretion of albumin is downregulated at 54 h in the combined presence of IL-6 (10 ng/mL) and IL-1β (10 ng/mL) (*p=0.047), as expected for this APP. Results are mean ± SD, n=3 replicates.



	Control	IL-1β	IL-6	IL-1β x IL-6
24 hr	0.2	1.0	4.8	5.2
48 hr	0.4	1.0	3.51	6.6

Fig 8. Monolayer C3A cell (1.3×10^5 cells/cm^2) secretion of IL-10 was increased by IL-6 and limited by IL-1β. Results are single replicates of pooled triplicate wells (left-24 h, right-48h for each pair of bars).

IL-1β, IL-6 and TNFα levels in subject plasma all trended downward 24 h after ELAD treatment (Fig 9), suggesting a shift from pro-inflammatory TH1-like profile to anti-inflammatory TH2 profile.

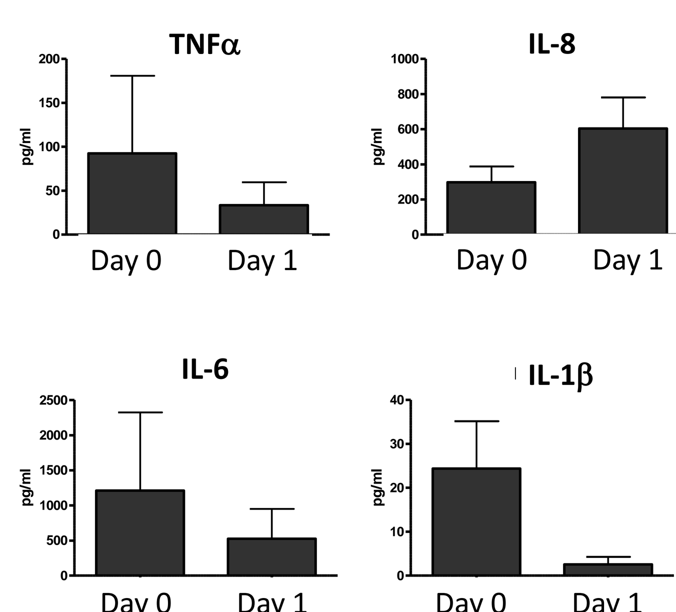


Fig 9. Measured plasma levels of TNFα, IL-8, IL-6 and IL-1β in subjects treated with the ELAD System at Day 0 (i.e. pre-treatment) and Day 1 (n=7 each). Differences were non-significant in each case (p>0.05).

RESULTS (cont.)

Oxidative burst (Fig 10) was significantly higher than normal plasma for neutrophils treated with pre-ELAD-treatment subject plasma and trended downward while on ELAD treatment and thereafter. Phagocytosis of *E. coli* (Fig 11) was lowest in neutrophils treated with pre-ELAD-treatment plasma and after 24 h of ELAD-treatment and increased in neutrophils treated with plasma from ELAD-treated subjects at the end of treatment and at 30-d follow-up.

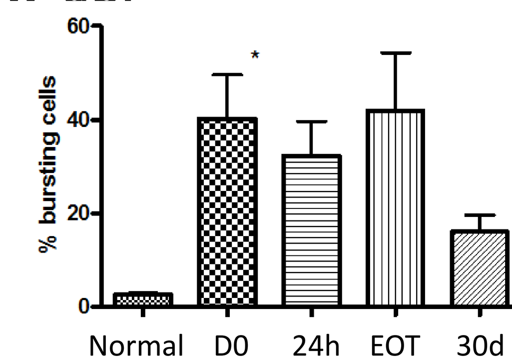


Fig 10. Resting oxidative burst of healthy donor neutrophils following incubation with normal plasma or plasma from ELAD-treated subjects collected at Day 0, 24 h post-treatment, end of treatment (EOT), or 30 d following treatment. *, p=0.03 for D0 vs 24h (Mann-Whitney paired comparison).

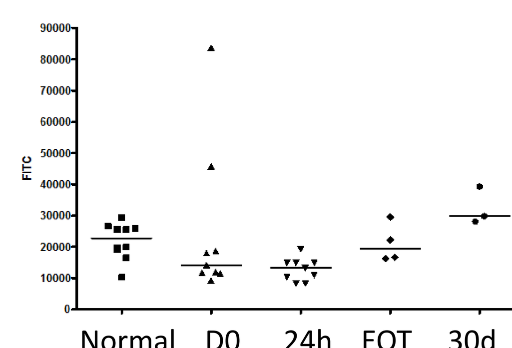


Fig 11. Phagocytic capacity of isolated neutrophils incubated with plasma from ELAD-treated subjects. Increased FITC fluorescence indicates more labelled *E.coli* have been consumed by the neutrophils. No significant differences were observed between time points.

Treating pro-inflammatory (M1) THP-1 cells with ELAD C3A cell cartridge conditioned medium reduced IL-1β pro-inflammatory cytokine secretion (Fig 12); however, ELAD CM had no affect on M1 secretion of IL-10 (Fig 13). Phagocytosis was greatest in M2 and unpolarized (M0) THP-1 cells. Phagocytosis capabilities were not conferred in the M1 THP-1 cells within 48 h of exposure to ELAD conditioned medium (data not shown).

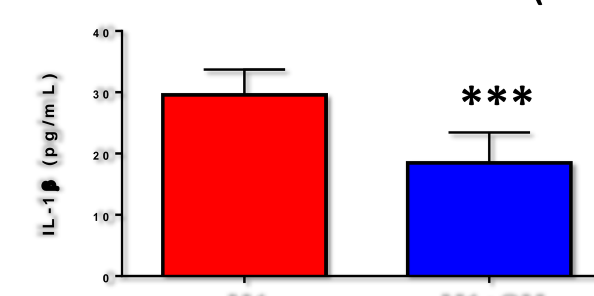


Fig 12. THP-1 M1 macrophage secretion of IL-1β is reduced in the presence of ELAD CM (*** p<0.001, Student's T-Test, n=6 biological replicates tested in duplicate).

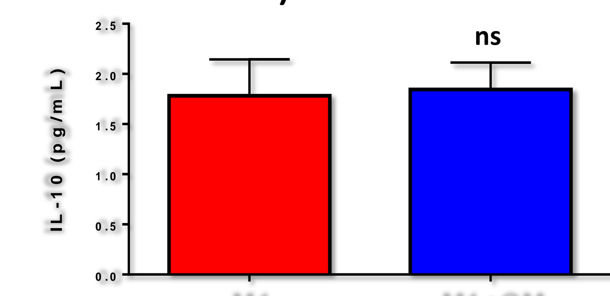


Fig 13. THP-1 M1 macrophage secretion of IL-10 is unaffected in the presence of ELAD CM (ns, p>0.05, Student's T-Test, n=6 biological replicates tested in duplicate).

CONCLUSIONS

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 TNFα, and to LPS was demonstrated by upregulated and/or constitutive expression of anti-inflammatory mediators such as IL-1Ra, AAT and IL-10.

Reduction of pro-inflammatory cytokines and increases in anti-inflammatory APPs in response to elevated cytokines and LPS in AH patients should contribute to resolution of inflammation and restoration of immune function. Preliminary evaluation of a small subset of ELAD-treated and control subjects supports this hypothesis.

These current studies demonstrate that VTL C3A cell-secreted factors can restore immune homeostasis and suggest a potential mechanism by which cell-based treatments, such as ELAD treatment, may provide benefit to AH patients.

ACKNOWLEDGEMENTS

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