Lyophilized Human Amnion and Chorion Membrane Modulates the Macrophage Inflammatory Response

Joseph Mikulin PhD, Sarah Moreno, Michelle Massee, and John R. Harper PhD

SAWC, May 2024

INTRODUCTION

A vigorous and efficient inflammatory response is required for progression through the wound healing cascade. Inflammatory dysregulation is pathomechanically linked to chronic inflammation and the failure of a wound to heal. The maternal-fetal interface is one of the most striking immunomodulatory microenvironments to be found in mammals. This immunomodulation may be leveraged therapeutically in wound healing through the application of amniotic tissue allografts. Therefore, this study aimed to determine whether and the degree to which lyophilized human amnion and chorion membrane (LHACM*) could modulate the response of macrophages to inflammatory stimulus *in vitro*.

MATERIALS AND METHODS

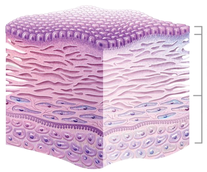

Figure 1. LHACM is a tri-layer allograft membrane. LHACM consists of human amnion, intermediate, and chorion placental layers. Placental tissue was prepared via the PURION‡ process to generate terminally sterilized, lyophilized tri-layer grafts.

Eluate Preparation: Human amniotic tissue (amnion and chorion layers) from 5 separate donors was prepared using the PURION®‡ process to produce LHACM. Soluble factors were then extracted via incubation in basal RPMI overnight at 4°C.

Cell Culture: Human monocytic THP-1 cells (ATCC TIB-202) and human T lymphoblast Jurkat cells (ATCC TIB-152) were maintained in RPMI medium with 10% fetal bovine serum. THP-1 monocytes were differentiated into macrophages (M0) using 10 ng/mL phorbol 12-myristate 13-acetate (PMA). M0 macrophages were incubated with 20 mg/mL LHACM for 48 hr to produce LHACM-elicited macrophages for downstream use in the efferocytosis assay.

Pro-inflammatory Response Measurement: To simulate M1 (pro-inflammatory) polarizing conditions, M0 macrophages were stimulated with 100 ng/mL LPS and 20 ng/mL interferon gamma (IFNγ) ± 1, 10, or 20 mg/mL LHACM eluate for 24 hr. Macrophage culture supernatants were harvested post-stimulation, and assays to measure soluble pro-inflammatory protein concentrations (Luminex) and Caspase-1-mediated inflammasome activity (Promega) were performed in accordance with manufacturers' instructions.

Efferocytosis: Bait cells were prepared by first fluorescently labeling Jurkat cells with CFSE, followed by incubating with staurosporine per the manufacturer's instructions (Cayman Chemical) for 5 hours to induce apoptosis. Labeled apoptotic bait cells were then washed and added to cultures of M0 or LHACM-elicited macrophages in complete RPMI at a 3:1 bait cell to effector cell ratio. After incubating overnight, macrophage cultures were washed and harvested for analysis by flow cytometry.

Flow cytometry: Single cell suspensions were stained with viability dye (Zombie NIR, BioLegend) and antibodies (BioLegend) against key macrophage markers CD36, CD80, CD86, CD163, CD206, and CD209. For measuring macrophage efferocytotic activity, macrophages co-incubated with CFSE-labeled apoptotic bait cells were harvested and analyzed. Data acquisition was performed using a 3 laser Cytek Northern Lights flow cytometer. Subsequent data analysis was performed using FlowJo software.

SUMMARY OF FINDINGS

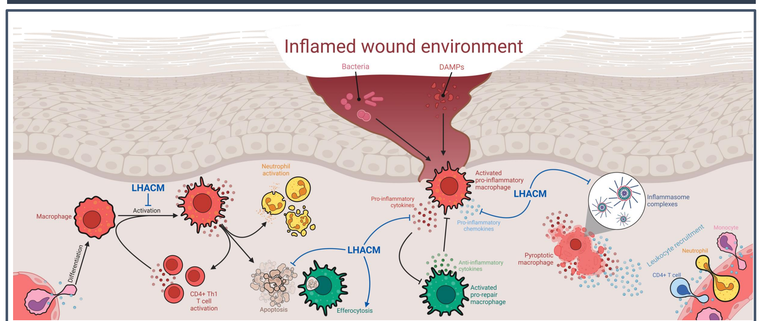

Figure 2. LHACM modulates multiple aspects of macrophage effector function. LHACM modifies complex macrophage biology both in the absence and presence of inflammatory stimulus. LHACM blunts pro-inflammatory macrophage polarization and promotes a blended macrophage surface phenotype. During pro-inflammatory challenge, LHACM protects macrophages from cell death, reduces the production of pro-inflammatory cytokines and chemokines, and dampens the activity of inflammasome effector protein Caspase-1. LHACM-elicited macrophages also demonstrate an increased rate of efferocytosis. These data suggest that LHACM may support the wound healing cascade through the modification of macrophage behavior, reducing the likelihood of inflammatory dysregulation and the development of related complications. This highlights a potential clinical mechanism by which LHACM may support the healing cascade and facilitate tissue repair. Created using BioRender.

RESULTS

LHACM elicits a blended macrophage surface phenotype

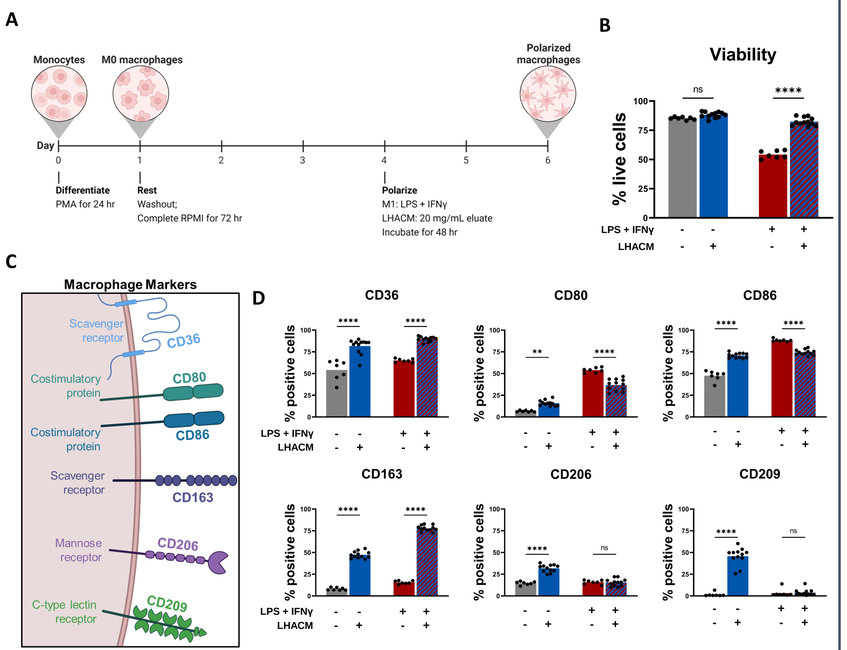

Figure 3. LHACM modifies macrophage polarization. (A) Macrophages were generated by treating THP-1 monocytes with 10 ng/mL PMA for 24 hr. Following a 72 hr rest period, macrophages were polarized for 48 hr with either 100 ng/mL LPS and 20 ng/mL IFNγ, 20 mg/mL LHACM, or both. Subsequent flow cytometry analysis was performed to assess the viability of resulting macrophages (B), as well as the expression of key macrophage markers (C) measured both by the frequency of expressing cells (D) and fluorescence intensity across all analyzed cells (E). ** $p \leq 0.01$ relative to control and **** $p \leq 0.0001$ relative to control based on a one-way ANOVA with a Tukey test; ns: statistically insignificant. Summary graphics (A and C) were generated using BioRender.

RESULTS

LHACM modulates the macrophage pro-inflammatory response

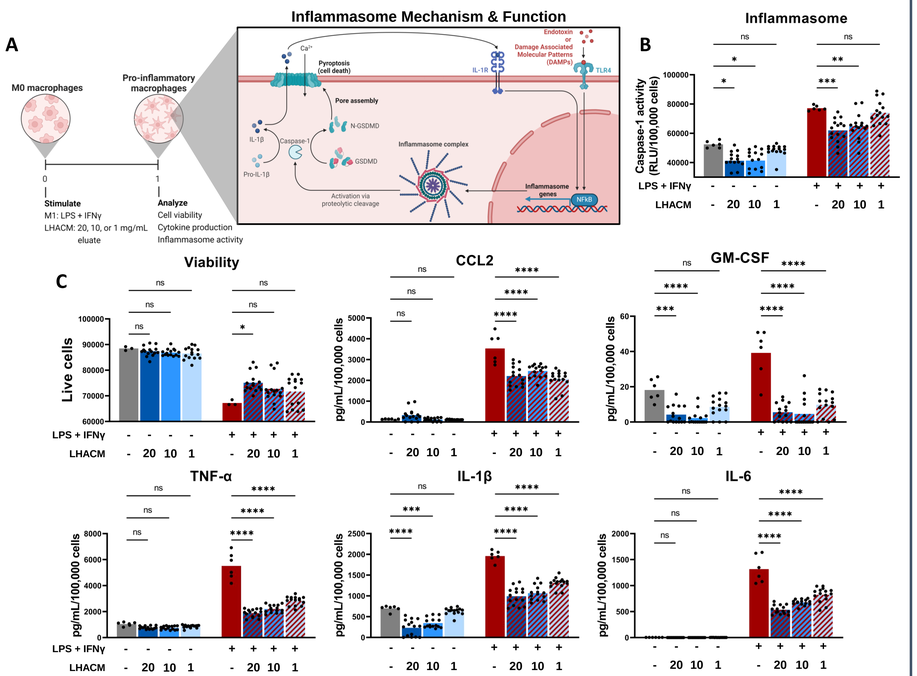

Figure 4. LHACM dampens the macrophage pro-inflammatory response. (A) To induce an inflammatory response and inflammasome activity, THP-1 M0 macrophages were stimulated for 24 hr with 100 ng/mL LPS and 20 ng/mL IFNγ, in the presence or absence of 20, 10, or 1 mg/mL LHACM. (B) Inflammasome function was determined by measuring the proteolytic activity of Caspase-1 in the supernatant against a cleavable Luciferase-linked reagent. (C) Viability and pro-inflammatory protein production were measured following stimulation via flow cytometry analysis of resulting cells and Luminex analysis of cell supernatants, respectively. * $p \leq 0.05$ relative to control, *** $p \leq 0.001$ relative to control, and **** $p \leq 0.0001$ relative to control based on a one-way ANOVA with a Tukey test; ns: statistically insignificant. Summary graphic (A) generated using BioRender.

LHACM increases macrophage efferocytosis

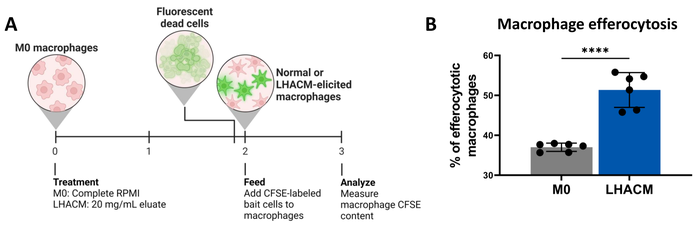

Figure 5. LHACM-elicited macrophages demonstrate increased efferocytotic activity. (A) THP-1 M0 macrophages were treated with 20 mg/mL LHACM for 48 hr before being washed and incubated with complete RPMI containing CFSE-labeled apoptotic bait cells. (B) The frequency of bait cell-containing macrophages was measured by flow cytometry. **** $p \leq 0.0001$ relative to M0 based on a T test. Summary graphic (A) was created using BioRender

All authors are employees of MIMEDX Group, Inc.

*EPIEFFECT®, MIMEDX Group, Inc. Marietta, GA; ‡PURION® Process, MIMEDX Group, Inc., Marietta GA