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Escherichia coli-induced temporal and differential secretion of heat-shock protein 70 and interleukin-1β by human fetal membranes in a two-compartment culture system

M. Osorio-Caballero [a], C. Perdigón-Palacio [b], G. García-López [c], O. Flores-Herrera [d], S. Olvera-Sánchez [d], I. Morales-Méndez [e], I. Sosa-González [e], J.F. Acevedo [f], A.M. Guzmán-Grenfell [b], A. Molina-Hernández [c], N.F. Díaz [c], H. Flores-Herrera [b, *]

[a] Department of Obstetrics and Gynecology, National Institute of Perinatology "Isidro Espinosa de los Reyes", Montes Urales #800, Col. Lomas de Virreyes cp, 11000 Mexico City, Mexico
[b] Department of Biochemistry and Molecular Biology, National Institute of Perinatology "Isidro Espinosa de los Reyes", Mexico City, Mexico
[c] Department of Cellular Biology, National Institute of Perinatology "Isidro Espinosa de los Reyes", Mexico City, Mexico
[d] Department of Biochemistry, School of Medicine, UNAM. Apdo. Postal 70-159, Copilco, Coyoacán, Mexico City, Mexico
[e] Department of Infectology and Immunology, National Institute of Perinatology "Isidro Espinosa de los Reyes", Mexico City, Mexico
[f] Department of Obstetrics and Gynecology, University of Texas SouthWestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235, USA

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ABSTRACT

Introduction: *Escherichia coli* is recognized as an etiological bacteria associated with chorioamnionitis and the preterm premature rupture of fetal membranes. This pathological condition induces pro-inflammatory cytokines and degradative metalloproteinases, which are considered biological markers secreted in an acute stage of infection. Heat-shock proteins (HSPs) are an important component of the innate immunity response and are found in different pathological conditions. They have not been previously measured in human fetal membranes in response to infectious conditions. We hypothesized that the choriodecidual tissue and amniotic epithelium secreted temporal and differential Hsp-60, Hsp-70, and interleukin (IL)-1β mediated by *E. coli* infection.
Methods: Fetal membranes were mounted in a two-compartment culture system and infected with two passes of live *E. coli* at different doses (10^2, 10^4, 10^5, and 10^6 colony-forming units (CFU)/mL) and intervals of incubation (3, 6, and 24 h). The culture medium was collected, and Hsp-60, Hsp-70, and IL-1β were assessed using the enzyme-linked immunosorbent assay (ELISA) method.
Results: After 3 and 6 h of infection, *E. coli* induced an increase in Hsp-70 secretion in the choriodecidual tissue. However, after 24 h of incubation, Hsp-70 was downregulated and we observed an increase in IL-1β secretion. By contrast, *E. coli* induced a lower Hsp-60 secretion in the amnion compared to Hsp-70.
Discussion: Human fetal membranes responded actively to *E. coli* infection, with an increase in Hsp-70 during the first hours of infection. After 24 h, there was an increase in the liberation of IL-1β.
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1. Introduction

Intrauterine infections are major etiological factors associated with preterm delivery (PTD) and premature rupture of the fetal membranes (PROM), and they predispose the neonate to a higher risk of intra-amniotic infection [1]. The early pathogenesis stages of intrauterine infections are not completely understood. However, much evidence has shown that fetal membranes actively respond to acute infection by secreting pro-inflammatory cytokines (interleukin (IL)-1β and IL-8); tumor necrosis factor alpha (TNF-α); chemokines (IL-8, monocyte chemotactic protein-1, and macrophage inflammatory protein-1) [2–4]; and degradative matrix metalloproteinases (MMP)-2, MMP-3, and MMP-9 [5,6]. These molecules are considered biological markers. Through these pro-inflammatory/degradative processes, the structure, stability, and

* Corresponding author. Department of Biochemistry and Molecular Biology, National Institute of Perinatology "Isidro Espinosa de los Reyes", Montes Urales #800, Col. Lomas de Virreyes cp, 11000 Mexico City, Mexico. Tel./fax: +52 55 55209705.
E-mail address: h.flores@inper.mx (H. Flores-Herrera).

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tensile force components of the extracellular matrix are modified, inducing PROM [2,6,7]. These biological markers are secreted by human fetal membranes in response to acute infections [8,9], and they cannot be used as markers in the initial stages of the infection.

Several lines of evidence suggest that heat-shock proteins (HSP) play an important role in normal and pathological human pregnancy [7,10]. Hsp-60, Hsp-70, and Hsp-90 have been detected by immunocytochemistry in the decidua and placenta during normal and preterm pregnancy [11,12]. HSPs are considered early signal molecules that lead to a cell danger response. This sensor system activates the professional cells (macrophages, monocytes, and dendritic cells) that trigger the immunological system response [13,14].

Escherichia coli (*E. coli*) is a gram-negative pathogenic bacteria associated with intra-amniotic infection and chorioamnionitis in pregnant women [15–17]. Hsp-70 is upregulated in response to vaginal and intra-amniotic infections associated with bacterial vaginosis [7,18,19]. Lipopolysaccharides (LPSs) induce the expression of Hsp-70 in human fetal membranes [20]. Additionally, Hsp-60 is upregulated during cellular stress and it induces the secretion of immunological cytokines [21,22].

However, the differential secretion of Hsp-60 and Hsp-70 by human fetal membranes in response to *E. coli* infection has not been studied. We postulate that Hsp-60, Hsp-70, and IL-1β are secreted by human fetal membranes in the initial stages of infection. The main objective of this study was to determine the *E. coli*-induced temporal profile secretion of Hsp-60, Hsp-70, and IL-1β by human fetal membranes in response to *E. coli* infection, using an in vitro two-compartment culture system model.

2. Material and methods

2.1. Mounting of fetal membranes in the Transwell system

Nine fetal membranes were collected after elective cesarean delivery. Patient consent and ethical approval were obtained before tissue collection, in accordance with the National Institute of Perinatology "Isidro Espinosa de los Reyes" guidelines. Women with uncomplicated, single, full-term (37–40 weeks) pregnancies who did not experience activation of labor, uterine contractions, or rupture of membranes were included in this study. All women underwent cesarean section. No evidence of microbiological signs of chorioamnionitis or lower genital tract infection was found.

Fetal membranes were transported to the laboratory in a sterile Dulbecco's modified Eagle medium (DMEM; Gibco, Bethesda, MD, USA) and rinsed in sterile Hank's balanced salt solution (Gibco) to remove adherent blood clots. Similar-sized samples (18 mm in diameter) were dissected and mounted in the Transwell culture system (Costar, New York, NY, USA). In this model, two compartments were created, choriodecidual (CHD; up face) and amnion (AM; down face). This model allows assessing the response simultaneously in the infection zone and in the opposite zone. A detailed description and validation of this model have been published previously [5]. The Transwell–fetal membrane was incubated in DMEM supplemented with 10% fetal calf serum (DMEM–FCS; Gibco) and an antibiotic–antimycotic solution (penicillin 100 U/mL, streptomycin 100 μg/mL; Gibco). Tissues were incubated at 37 °C with 5% CO_2 and 95% air for 18 h.

After the incubation period, the explants were washed twice with a sterile saline solution to remove DMEM–FCS. To each compartment, 1 mL of DMEM with 0.2% lactalbumin hydrolysate (DMEM–LHA; Gibco) was added.

2.2. Bacterial strain and preparation

E. coli clinical strain was isolated from the blood of a newborn whose mother had signs and symptoms of chorioamnionitis, and was diagnosed with PROM (<35 weeks). This strain was characterized at the Infectology Department of the National Institute of Perinatology. Bacteria were grown in 5% sheep blood agar (DIBICO, Mexico City, Mexico) supplemented with 10% base tryptic soy agar (DIBICO) for 2 days. Bacterial colonies were selected with bacteriological instruments and deposited in sterile phosphate-buffered saline (PBS) (1×) solution.

2.3. Human fetal membrane infection

The explants were infected in duplicate, and three approaches were followed: (1) control (basal conditions), (2) CHD, and (3) AM. In each case, the membranes were infected after a second pass with live *E. coli* in serial dilutions (10^2, 10^4, 10^5, and 10^6 colony-forming units (CFU)/mL). The CFU was based on a turbidity equivalent to 0.5 McFarland standard. The explants were infected at intervals of 3, 6, and 24 h.

After this time, medium from each compartment was collected using sterile transfer pipettes (Falcon, Becton–Dickinson, Franklin Lakes, NJ, USA). The samples were immediately centrifuged at 5000 rpm for 3 min at 4 °C. The bacterial sediment was discarded and the supernatants stored at −70 °C until assayed.

2.4. Viability of the human fetal membranes

All explants were washed twice with sterile saline solution to remove DMEM–LHA and *E. coli*. The CHD and AM compartments were treated with 200 μL of a 5-mg/mL XTT (sodium 3′-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6 nitro) benzene sulfonic acid hydrate) (Roche Diagnostics GmbH; Mannheim, Germany) labeling reagent and 50 μL of electron-coupling reagent (PMS; N-methyl dibenzopyrazine methyl sulfate) for a 1-h incubation period.

Carbonyl cyanide m-chlorophenylhydrazone (CCCP; Sigma–Aldrich, St Louis, MO, USA), a mitochondrial uncoupler, was used as a positive control. CCCP was dissolved in dimethyl sulfoxide at a concentration of 80 μM [23]. The spectrophotometrical absorbance of the samples was measured by a Benchmark plus (model 550; BioRad, Hercules, CA, USA) microplate reader. The wavelength to measure the absorbance of the formazan product was 475 nm.

2.5. Measurement of HSP and IL-1β in conditioned medium

Hsp-60, Hsp-70, and IL-1β secreted from either the CHD or AM compartments were quantified using commercially available enzyme-linked immunosorbent assay (ELISA), using a specific Douset (R&D system, Minneapolis, MN, USA) kit, as previously described by Flores-Herrera et al. [5].

For Hsp-60 (Cat. No. DYC1800-2) and Hsp-70 (Cat. No DYC1663-2), a standard curve was developed from 1.25 to 80 ng/mL and from 312.5 to 20,000 pg/mL, respectively. In the case of IL-1β (Cat. No. DY201), a standard curve was developed from 4 to 260 pg/mL. The sensitivity was 2.0 pg/mL [5]. The values were reported as picograms per milliliter.

2.6. Statistical analysis

The data for each infection zone were analyzed using Friedman repeated measures analysis of variance (5 (concentration of bacteria) × 3 (exposure time)). Post hoc comparisons were performed with pairwise multiple comparison procedures (Tukey's test). The software used was SigmaPlot for Windows Version 11.0. All values are presented as mean ± standard error of the mean (SEM).

3. Results

3.1. Human fetal membrane viability assay

3.1.1. CHD viability

The viability in the CHD compartment was determined at 3 (Fig. 1A), 6 (Fig. 1B), and 24 h (Fig. 1C) postinfection, as well as at basal condition (Fig. 1). In all experimental conditions, no statistical difference was observed between basal groups in comparison with *E. coli* postinfection groups. As we expected, the metabolic activity of CHD explants was reduced by the proton translocator (CCCP), as compared with the control (Fig. 1).

3.1.2. Amniotic viability

As in the CHD compartment, the viability of the AM compartment was determined at 3 (Fig. 2A), 6 (Fig. 2B), and 24 h (Fig. 2C) postinfection and at basal condition. As in the CHD compartment, no statistical difference was observed between the basal groups and *E. coli* postinfection groups ($p = 0.33$). Furthermore, CCCP reduced the metabolic activity of fetal membranes compared to the control group (Fig. 2).

These results indicate that temporal infection has no effect on the viability of human fetal membranes. We then investigated the secretion of HSPs by fetal membrane compartments exposed to *E. coli*.

3.2. E. coli-induced production of HSP in human fetal membranes

We determined the secretion profile of Hsp-60 and Hsp-70 in the CHD or AM compartments after 3, 6, and 24 h of infection in in vitro conditions.

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