Journal of Reproductive Immunology 109 (2015) 24–30

Contents lists available at ScienceDirect

Journal of Reproductive Immunology

journal homepage: www.elsevier.com/locate/jreprimm

Blood SC5b-9 complement levels increase at parturition during term and preterm labor

Enrique Segura-Cervantes[a], Javier Mancilla-Ramirez[b], Luis Zurita[c], Yuriria Paredes[d], José Luis Arredondo[e], Norma Galindo-Sevilla[a,*]

[a] Departamento de Infectología e Inmunología Perinatal, Instituto Nacional de Perinatologia (INPer), Mexico
[b] Escuela Superior de Medicina, IPN, Mexico
[c] División Académica de Ciencias de la Salud, UJAT, Mexico
[d] Departmento de Biología Celular, INPer, Mexico
[e] Unidad de Investigación Clínica, INP, Mexico

ARTICLE INFO

Article history:
Received 20 September 2014
Received in revised form 17 February 2015
Accepted 19 February 2015

Keywords:
Complement
Pregnancy
Parturition
Labor
Fetal membranes

ABSTRACT

We explored the hypothesis that complement, an innate and adaptive immune effector, is active in the plasma of parturient women and is deposited on fetal membranes collected after delivery. A cross-sectional study was designed to evaluate complement activity at parturition. Pregnant women ($n = 97$) between 15 and 41 years of age were enrolled in a hospital protocol during the perinatal period to assess both SC5b-9 complement activity in blood and complement deposition on fetal membranes during parturition. Soluble SC5b-9 complement activity in plasma fractions was measured using a standard enzyme-linked immunosorbent assay (ELISA) that included specific anti-complement antibodies. Complement deposition on membranes was analyzed using immuno-dot blots and immunohistochemistry. Soluble SC5b-9 complement complex levels were increased in the plasma of women during term labor (TL; median 3361; range 1726–5670 ng/mL), preterm labor (PL; median 2958; range 1552–7092 ng/mL), and preterm premature rupture of membranes (PPROM; median 2272; range 167–6540 ng/mL) compared with pregnant women who were not in labor (P; median 1384; range 174–4570 ng/mL; $P < 0.001$, Kruskal–Wallis test). Active complement, as assessed by the C9 neo-antigen in C5b-9 complexes, was deposited on fetal membranes, with no difference between term and preterm delivery. The deposition of active complement on fetal membranes was confirmed by immunohistochemistry. Women who underwent non-labor-indicated Cesarean sections did not exhibit complement deposition. Soluble SC5b-9 complement complex levels increased in the plasma of women during parturition, and complement C5b-9 complexes were deposited on fetal membranes.

* Corresponding author at: Departamento de Infectología e Inmunología Perinatal, Instituto Nacional de Perinatología, Montes Urales #800, Col. Lomas de Virreyes, Mexico City 11000, Mexico. Tel.: +52 55 55 20 99 00x103.
E-mail addresses: enriquesc2020@terra.com.mx (E. Segura-Cervantes), javiermancilla@hotmail.com (J. Mancilla-Ramirez), Drluiszurita@gmedico.com (L. Zurita), yuriparedes@hotmail.com (Y. Paredes), arredondo.joseluis@gmail.com (J.L. Arredondo), NGalindoSevilla@hotmail.com (N. Galindo-Sevilla).

http://dx.doi.org/10.1016/j.jri.2015.02.008
0165-0378/

1. Introduction

Parturition is a physiological process that requires the synchronization of uterine contractions, cervical dilatation, and membrane rupture. Several reviews have emphasized that the physiological phenomena, molecules, and cellular mechanisms that drive the process of human parturition are not completely understood (Breuiller-Fouche and Germain, 2006). The complement system is an important effector of the immune system because it forms pores in cell membranes that lead to cell lysis; thus, the complement system is thought to play a crucial role during parturition. C4B and C1R protein levels are lower during spontaneous vaginal delivery (SVD) than during pre-labor in the same women (Yuan et al., 2012). However, the complement system and its role during parturition have not been explored as extensively as the cytokines that mediate the parturition-dependent inflammatory response. New techniques, including standard enzyme-linked immunoabsorbent assay (ELISA), now permit the evaluation of complement activity with improved sensitivity. Different methodological approaches have been used to determine complement activity in biological fluids, such as those used to assess the protein complex C5b-9 in plasma. This protein complex is a common mediator of cell lysis that is generated from any of the four complement activation pathways (Huber-Lang et al., 2006). The regulatory S protein binds to C5b-9, forming the SC5b-9 complex, which is soluble and can be detected in plasma by ELISA (Mollnes et al., 1985).

We hypothesized that the complement system is active at parturition during established labor. This study aimed to investigate whether the active form of complement, C5b-9, is elevated in plasma as SC5b-9 at parturition and whether complement is deposited on fetal membranes.

2. Materials and methods

2.1. Study design

To understand complement activity at parturition, a cross-sectional study was designed to assess complement activity in the plasma and complement deposition on fetal membranes. Pregnant women ($n = 97$) between 15 and 41 years of age who were treated as outpatients from 12 weeks of gestation until delivery at the National Institute of Perinatology (INPer, Mexico City, Mexico) were recruited for the study. Patients with cancer and patients receiving immunotherapy for transplants or autoimmunity or current steroid treatment were excluded from the study. The patients provided written informed consent during their clinical check-ups. Each patient consented to donate four fetal membrane samples (1 cm in diameter) after childbirth. Furthermore, one 3-mL sample of whole peripheral blood was obtained from each patient at delivery or during normal examinations as stated in Section 3. The study participants included pregnant women (P) at <37 weeks of gestational age. Sample membranes were also isolated from women undergoing an indicated Cesarean section at <36 weeks. Women at >37 weeks of gestational age comprised the term labor (TL) group, and women at <37 weeks of gestational age were assigned to one of two subgroups: the preterm labor (PL) or preterm premature rupture of membranes (PPROM) group. A flow diagram is presented in Fig. 1. The study participants were at minimal risk. The study was approved by the research and bioethical committees of the National Institute of Perinatology and was in compliance with international guidelines for human studies.

2.2. SC5b-9 protein complex assessment

The C5b-9 complex binds the regulatory S protein after the C5b-7 protein complex is properly assembled in the plasma (Mollnes et al., 1985; Bhakdi and Tranum-Jensen, 1982). SC5b-9 complex levels in the plasma are indicative of complement activation, which causes subsequent cell lysis. SC5b-9 complex levels were analyzed using a standard ELISA assay (Quidel, Mountain View, CA, USA) as previously reported (Walter et al., 2010). Participant plasma samples were routinely diluted 1:40. The samples were assayed in triplicate wells (100 μL) and were incubated for 60 min according to the manufacturer's instructions. The method was quantitative for SC5b-9 complex levels ranging from 20 to 150 ng/mL. The color was developed by incubating the samples with 100 μL/well of the chromogenic substrate OPD (*O*-phenylenediamine dihydrochloride, Sigma-Aldrich, St. Louis, MO, USA) for 30 min at room temperature. The plates were read using an automated ELISA plate reader (Synergy 2, Bio-Tek Instruments, Winooski, VT, USA) after the enzyme reaction was terminated by the addition of 50 μL of 0.1 M H_2SO_4. The absorbance was measured at 405 nm.

2.3. Fetal membrane sampling

We investigated whether active complement was deposited on fetal membranes (1 cm in diameter), including the chorion and amnion. The fetal membranes were obtained within the first 30 min after childbirth and were assessed in duplicate at four points from the rupture site following a previously published protocol (McLaren et al., 1999)Ref. . One membrane sample from each duplicate was incubated in phosphate-buffered saline (PBS; pH 7.4) containing a mammalian protease inhibitor cocktail (PIC, 1:50 dilution; Sigma-Aldrich, St. Louis, MO, USA). The samples were frozen at −70 °C and stored until they were used in standard immuno-dot blot assays (Janyapoon et al., 2000) of membrane complement components. The other membrane samples were used for the immunohistochemical detection of complement components.

2.4. Protein membrane extraction and immuno-dot blot assay

Complement components were detected in protein fractions extracted from fetal membranes. Briefly, the membrane tissue was mechanically disrupted in a tissue grinder with 3 mL of cold PBS containing a protease inhibitor cocktail (PBS-PIC). The suspended cells were recovered and centrifuged at 400 × *g*. The cell pellet was