Received: 8 July 2016 | Accepted: 15 August 2016

DOI: 10.1111/aji.12562

SHORT COMMUNICATION

WILEY AJRI American Journal of Reproductive Immunology

In vivo T-cell activation by a monoclonal αCD3ε antibody induces preterm labor and birth

Nardhy Gomez-Lopez[1,2,3] | Roberto Romero[1,4,5,6] | Marcia Arenas-Hernandez[1,2,7] | Hyunyoung Ahn[1,2] | Bogdan Panaitescu[1,2] | Felipe Vadillo-Ortega[8] | Carmen Sanchez-Torres[7] | Katherine S. Salisbury[2] | Sonia S. Hassan[1,2]

[1]Perinatology Research Branch, NICHD/NIH/DHHS, Bethesda, MD, and Detroit, MI, USA

[2]Department of Obstetrics & Gynecology, Wayne State University School of Medicine, Detroit, MI, USA

[3]Department of Immunology & Microbiology, Wayne State University School of Medicine, Detroit, MI, USA

[4]Department of Obstetrics & Gynecology, University of Michigan, Ann Arbor, MI, USA

[5]Department of Epidemiology & Biostatistics, Michigan State University, East Lansing, MI, USA

[6]Center for Molecular Obstetrics & Genetics, Wayne State University, Detroit, MI, USA

[7]Department of Molecular Biomedicine, CINVESTAV, Mexico City, Mexico

[8]Unit of Vinculation, Faculty of Medicine, Universidad Nacional Autónoma de México en el Instituto Nacional de Medicina Genómica, Mexico City, Mexico

Correspondence
Nardhy Gomez-Lopez, Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, MI, USA and Perinatology Research Branch, NICHD/NIH/DHHS, Detroit, MI, USA.
Email: nardhy.gomez-lopez@wayne.edu
Roberto Romero, Perinatology Research Branch, NICHD/NIH/DHHS, Detroit, MI, USA and Wayne State University/Hutzel Women's Hospital, Detroit, MI, USA.
Email: romeror@mail.nih.gov

Problem: Activated/effector T cells seem to play a role in the pathological inflammation associated with preterm labor. The aim of this study was to determine whether in vivo T-cell activation by a monoclonal αCD3ε antibody induces preterm labor and birth.

Method of study: Pregnant B6 mice were intraperitoneally injected with a monoclonal αCD3ε antibody or its isotype control. The gestational age, the rates of preterm birth and pup mortality at birth as well as the fetal heart rate and umbilical artery pulsatility index were determined.

Results: Injection of a monoclonal αCD3ε antibody led to preterm labor/birth (αCD3ε 83 ± 16.97% [10/12] vs isotype 0% [0/8]) and increased the rate of pup mortality at birth (αCD3ε 87.30 ± 8.95% [77/85] vs isotype 4.91 ± 4.34% [3/59]). In addition, injection of a monoclonal αCD3ε antibody decreased the fetal heart rate and increased the umbilical artery pulsatility index when compared to the isotype control.

Conclusion: In vivo T-cell activation by a monoclonal αCD3ε antibody in late gestation induces preterm labor and birth.

KEYWORDS
adaptive immunity, cytokines, maternal–fetal rejection, mouse, parturition, pregnancy, T cells

1 | INTRODUCTION

Preterm birth delivery before 37 weeks of gestation is the leading cause of perinatal morbidity and mortality worldwide. Approximately 70% of all preterm births occur after spontaneous preterm labor,[1] a syndrome of multiple etiologies.[2] Pathological inflammation is implicated in the process of preterm parturition[3–5] and can result from the activation of innate[6–12] or adaptive immunity.[13,14] Among adaptive immune cells, T cells are implicated in the mechanisms that lead to spontaneous labor at term[15–17] and spontaneous preterm labor.[13,14,18]

T cells are adaptive immune cells critical for antigen-specific immunity as well as for defense against future infections. The defining feature of T cells is the T-cell receptor (TCR), which allows them to perform most of their antigen-specific functions through interactions with MHC class I and class II molecules. T-cell subsets include: (i) CD4+ T helper (Th) cells, which respond to exogenous antigens presented through MHC class II signaling;[19–23] and (ii) CD8+ cytotoxic T cells or CTLs, which are involved in the lysis of aberrant cells and respond to endogenous antigens or self-recognition through MHC class I signaling.[19,20,23] Discrimination of self- and non-self,[24] along with the concept of tolerance,[25–27] are two of the most clinically important aspects of T-cell functionality, as even slight errors in either process can lead to diseases such as autoimmune disorders. T cells are activated through the engagement of the TCR and co-stimulation.[28] Upon activation, effector T cells secrete cytokines that can promote their proliferation and the activation of T-cell-dependent B cells as well as regulate the activity of innate immune cells such as macrophages.[28] In vivo T-cell activation is achieved by administering low concentrations (4–10 μg) of a monoclonal αCD3ε antibody (e.g., clone 145-2C11) .[29,30] This antibody recognizes the CD3ε molecule and activates T cells in the absence of antigen, as it evades the TCR antigen-specific recognition mechanism.[31,32] Herein, we hypothesized that the administration of a monoclonal αCD3ε antibody (clone 145-2C11) in late gestation will cause pathological inflammation by initiating innate and adaptive immune responses which, in turn, could lead to preterm labor and birth.

The aim of this study was to determine whether in vivo T-cell activation by a monoclonal αCD3ε antibody induces preterm labor and birth. Also, we examined whether administration of this antibody would cause fetal death or fetal compromise using Doppler ultrasound.

2 | MATERIALS AND METHODS

2.1 | Animals

C57BL/6J (B6) mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA) and bred in the animal care facility at the C.S. Mott Center for Human Growth and Development at Wayne State University, Detroit, Michigan, USA. Mice were housed under a circadian cycle (light: dark=12:12 hour). Eight- to 12-week-old females were mated with males of proven fertility. Females were examined daily between 8:00 AM. and 9:00 AM. for the presence of a vaginal plug, which indicated 0.5 days *post coitum* (dpc). Upon observation of a vaginal plug, females were housed separately from males, their weight was monitored, and a gain of two or more grams by 12.5 dpc confirmed pregnancy. Procedures were approved by the Institutional Animal Care and Use Committee at Wayne State University (Protocol No. A 09-08-12).

2.2 | Intraperitoneal administration of a monoclonal αCD3ε antibody

Pregnant B6 mice were intraperitoneally injected with 10 μg of a purified anti-mouse CD3ε (αCD3ε) antibody (BD Biosciences, San Jose, CA, USA, Clone 145-2C11; n=12) dissolved in 200 μL of sterile 1X phosphate-buffered saline (PBS) on 16.5 dpc. Controls were injected with 10 μg of the isotype control (IgG1 κ Isotype; BD Biosciences, Clone A19-3; n=8) dissolved in 200 μL of sterile PBS on 16.5 dpc. Following injection, mice were monitored using a video camera with an infrared light (Sony Corporation, Tokyo, Japan) until delivery.

2.3 | Outcome variables

Preterm labor/birth was defined as delivery occurring before 18.0 dpc, and its rate was represented by the percentage of females delivering preterm among those delivering at term (19.5 ± 0.5 dpc). Gestational age was defined as the time elapsed from the detection of the vaginal plug (0.5 dpc) through the delivery of the first pup. The rate of pup mortality at birth was defined as the percentage of pups found dead among the total litter size.

2.4 | In vivo imaging by ultrasound

Pregnant B6 mice were intraperitoneally injected with a monoclonal αCD3ε antibody or its isotype control on 16.5 dpc (n=12–13 each). Sixteen hours post-injection (prior to preterm labor/birth in mice injected with αCD3ε) ultrasound was performed, as previously described.[33,34] Mice were anesthetized by inhalation of 2%–3% of isoflurane (Aerrane; Baxter Healthcare Corporation, Deerfield, IL, USA) and 1–2 L/min of oxygen in an induction chamber. Anesthesia was maintained with a mixture of 1.5%–2% of isoflurane and 1.5–2 L/min of oxygen. Mice were positioned on a heated platform and stabilized using adhesive tape. Fur was removed from the abdomen and thorax following the application of Nair cream (Church & Dwight Co., Inc., Ewing, NJ, USA) to those areas. Body temperature was maintained at 37 ± 1°C and monitored using a rectal probe. Respiratory and heart rates were monitored by electrodes embedded in the heated platform. An ultrasound probe was fixed and mobilized with a mechanical holder, and the transducer was slowly moved toward the abdomen. The fetal heart rate and umbilical artery pulsatility index (PI) were examined with the 55-MHz linear ultrasound probe (VisualSonics Inc., Toronto, ON, Canada). Umbilical artery PI was calculated using the following formula: PI=(systolic velocity − diastolic velocity)/mean velocity. Ultrasound signals were processed, displayed, and stored using the Vevo Imaging Station (VisualSonics Inc). Following ultrasound, females were placed under a heat lamp for recovery, which occurred 10–20 minutes after heating.

2.5 | Statistical analysis

Statistical analyses were performed using SPSS, Version 19.0 (IBM Corporation, Armonk, NY, USA). The following tests were performed to compare differences between the groups: a Fisher's exact test for the rates of preterm labor/birth, a Mann-Whitney *U* test for gestational age, a logistic regression model for the rates of pup mortality at birth, and *t* tests for fetal heart rate and umbilical artery PI. A *P* value of .05 was considered statistically significant. When

proportions are displayed, percentages and 95% confidence intervals (CI) are shown. Medians are shown with the interquartile range (IQR), and means are shown with the standard error of the mean (SEM).

3 | RESULTS

The frequency of preterm labor/birth after an intraperitoneal injection of a monoclonal αCD3ε antibody was higher than that following an intraperitoneal injection of its isotype control (αCD3ε 83 ± 16.97% [10/12] vs isotype 0% [0/8]; *P*<.0001; Fig. 1a). Pregnant mice injected with a monoclonal αCD3ε antibody had a shorter gestational age than those injected with the isotype control (αCD3ε 17.51 dpc [IQR=17.46–17.59 dpc] vs isotype 19.19 dpc [IQR=19.03–19.28 dpc]; *P*=.002; Fig. 1b). Intraperitoneal injection of a monoclonal αCD3ε antibody was also associated with an increased rate of pup mortality at birth (αCD3ε 87.30 ± 8.95% [77/85] vs isotype 4.91 ± 4.34% [3/59]; *P*<.0001; Fig. 1c).

Most of the dams injected with a monoclonal αCD3ε antibody delivered premature non-viable pups (Fig. 1c). We then investigated whether T-cell activation was causing fetal death (i.e., fetuses without a heartbeat) or fetal compromise (i.e., fetuses with abnormal umbilical artery velocimetry and fetal heart rate[35,36]). Therefore, Doppler ultrasound was performed (Fig. 2a,b) prior to preterm labor/birth in mice injected with a monoclonal αCD3ε antibody or matched-time isotype controls. Fetuses from dams injected with a monoclonal αCD3ε antibody were viable, as a heartbeat was detected (Fig. 2a). However, these fetuses were bradycardic when compared to the controls (αCD3ε 104.32 bpm [SEM ± 4.11 bpm; n=88] vs isotype

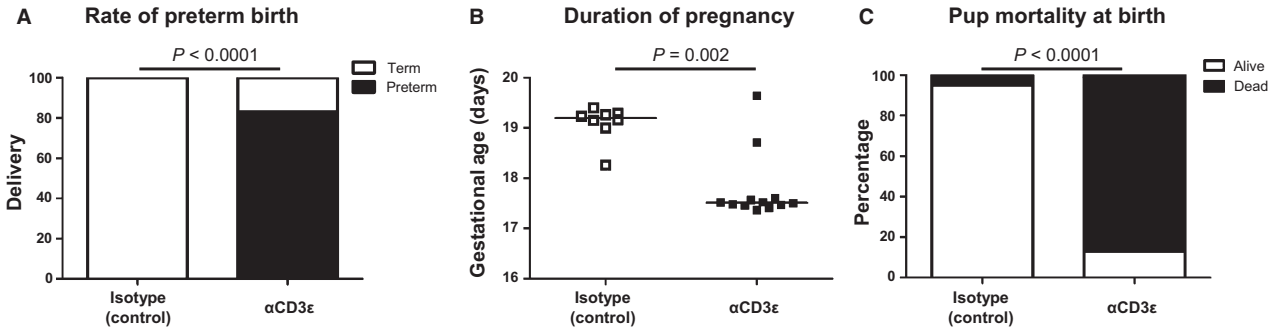

FIGURE 1 Intraperitoneal injection of a monoclonal αCD3ε antibody. Pregnant B6 mice were intraperitoneally injected with a monoclonal αCD3ε antibody (10 μg dissolved in 200 μL of sterile 1X phosphate-buffered saline [PBS]; n=12) on 16.5 d *post coitum* (dpc). Control mice were injected with an isotype (10 μg dissolved in 200 μL of sterile PBS; n=8) on 16.5 dpc. The rate of preterm labor/birth (a), gestational age (b), and rate of pup mortality at birth (c) are displayed

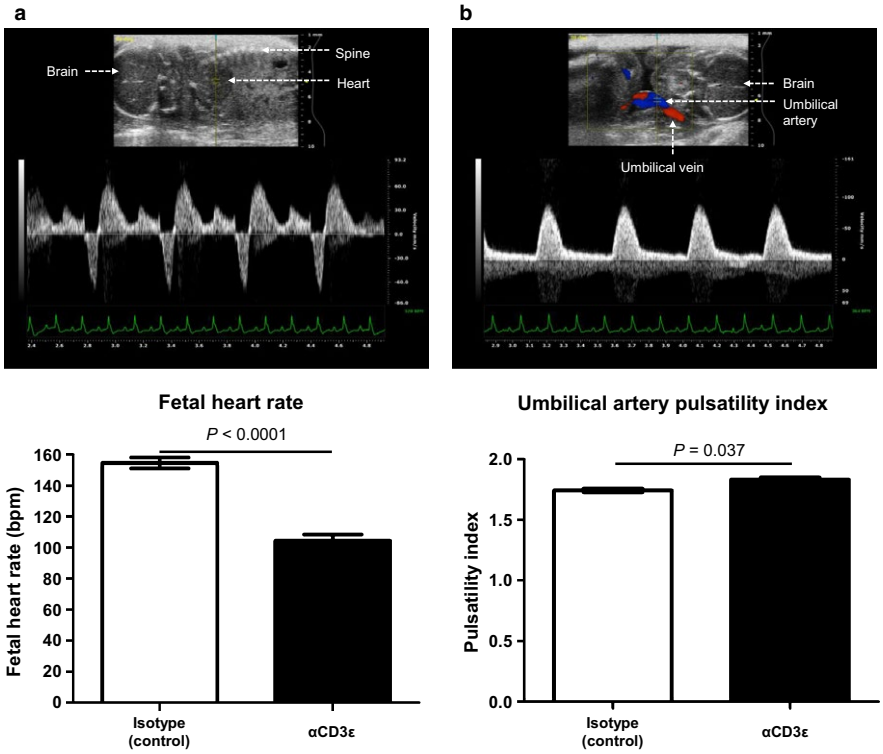

FIGURE 2 In vivo imaging by Doppler ultrasound. Doppler ultrasound was performed on fetuses prior to preterm labor/birth in dams injected with a monoclonal αCD3ε antibody (10 μg dissolved in 200 μL of sterile 1X phosphate-buffered saline [PBS]; n=13) or time-matched isotype controls (10 μg dissolved in 200 μL of sterile PBS; n=12). Fetal heart rate (a) and umbilical artery pulsatility index (b) were recorded. Data are from 12 to 13 independent litters

154.69 bpm [SEM ± 3.54 bpm; n=82]; *P*<.0001; Fig. 2a). Fig. 2b shows how Doppler ultrasound was used to determine the blood flow through the umbilical artery, which was used to calculate the pulsatility index. Fetuses from dams injected with a monoclonal αCD3ε antibody had an increased umbilical artery PI when compared to the controls (αCD3ε 1.83 PI [SEM ± 0.01 PI; n=87] vs isotype 1.74 PI [SEM ± 0.01 PI; n=82]; *P*=.037; Fig. 2b). Together, these data demonstrated that, although pups from dams injected with a monoclonal αCD3ε antibody did not die in the uterus, their health was compromised before birth.

4 | DISCUSSION

T cells have been implicated in the mechanisms that lead to spontaneous labor at term[15–17] and spontaneous preterm labor.[13,14,18] In the study herein, we demonstrated for the first time that the intraperitoneal injection of a monoclonal αCD3ε antibody induces preterm labor and birth. Administration of this antibody causes a massive systemic release of several T-cell-derived cytokines such as tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-2, and IL-3.[37] These data suggest that T-cell activation causes a systemic inflammatory response in the mother, leading to preterm labor and birth.

Activated/effector CD8+ T cells (CTLs) and, to a lesser extent, CD4+ T cells, are observed in chronic inflammatory lesions of the placenta, such as villitis of unknown etiology (VUE).[38–40] CTLs are also abundant in the endometrium[41] and cervix[42] of premenopausal women, as well as in the systemic circulation[13] and chorioamniotic membranes of patients with chronic chorioamnionitis,[43] the most common placental lesion in late spontaneous preterm birth.[44] These cytotoxic T cells induce trophoblast apoptosis and damage the integrity of the chorioamniotic membranes[14,43] which, in turn, may induce the premature rupture of these tissues and consequently lead to labor. Activated/effector T cells also mediate allograft rejection; indeed, both VUE and chronic chorioamnionitis are considered histopathologic manifestations of T-cell-mediated rejection of the semi-allograft fetus.[14] Altogether, these data led us to propose that in vivo T-cell activation represents a preterm birth model of maternal–fetal T-cell mediated rejection.

In vivo T-cell activation caused fetal compromise by inducing bradycardia and altering the umbilical artery pulsatility index. This finding is consistent with two facts: (i) VUE is associated with an abnormal Doppler velocimetry of the umbilical artery;[45] and (ii) chronic chorioamnionitis is associated with fetal death.[46] The negative effects of T-cell activation on the fetal heart rate are most likely mediated by TNF-α and IL-2 (T-cell cytokines), which induce cardiomyopathy.[47,48] Taken together, these data suggest that in vivo T-cell activation induces fetal compromise by causing fetal inflammatory response syndrome (FIRS), of which maternal–fetal rejection may be the mechanism of disease (i.e., FIRS type 2).[14]

In summary, the study herein provides evidence that activation of maternal T cells by a monoclonal αCD3ε antibody induces fetal compromise and the premature expulsion of the semi-allograft fetus.

ACKNOWLEDGMENTS

This research was supported, in part, by the Perinatology Research Branch, Division of Intramural Research, *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, U. S. Department of Health and Human Services (NICHD/NIH/DHHS), and, in part, with federal funds from the NICHD/NIH/DHHS under Contract No. HHSN275201300006C. This research was also supported by the Wayne State University Perinatal Initiative in Maternal, Perinatal and Child Health. We thank Tara N. Mial for her critical readings of the manuscript.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

REFERENCES

1. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet*. 2008;371:75–84.
2. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. *Science*. 2014;345:760–765.
3. Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel L, Hassan S. The role of inflammation and infection in preterm birth. *Semin Reprod Med*. 2007;25:21–39.
4. Romero R, Espinoza J, Kusanovic JP, et al. The preterm parturition syndrome. *BJOG*. 2006;113(Suppl. 3):17–42.
5. Romero R, Espinoza J, Goncalves LF, Kusanovic JP, Friel LA, Nien JK. Inflammation in preterm and term labour and delivery. *Semin Fetal Neonatal Med*. 2006;11:317–326.
6. Koga K, Cardenas I, Aldo P, et al. Activation of TLR3 in the trophoblast is associated with preterm delivery. *Am J Reprod Immunol*. 2009;61:196–212.
7. Cardenas I, Means RE, Aldo P, et al. Viral infection of the placenta leads to fetal inflammation and sensitization to bacterial products predisposing to preterm labor. *J Immunol*. 2010;185:1248–1257.
8. Cardenas I, Mulla MJ, Myrtolli K, et al. Nod1 activation by bacterial iE-DAP induces maternal-fetal inflammation and preterm labor. *J Immunol*. 2011;187:980–986.
9. Romero R, Chaiworapongsa T, Alpay Savasan Z, et al. Damage-associated molecular patterns (DAMPs) in preterm labor with intact membranes and preterm PROM: a study of the alarmin HMGB1. *J Matern Fetal Neonatal Med*. 2011;24:1444–1455.
10. Koga K, Izumi G, Mor G, Fujii T, Osuga Y. Toll-like receptors at the maternal-fetal interface in normal pregnancy and pregnancy complications. *Am J Reprod Immunol*. 2014;72:192–205.
11. Kim YM, Romero R, Chaiworapongsa T, et al. Toll-like receptor-2 and -4 in the chorioamniotic membranes in spontaneous labor at term and in preterm parturition that are associated with chorioamnionitis. *Am J Obstet Gynecol*. 2004;191:1346–1355.
12. Lappas M. Cellular inhibitors of apoptosis proteins cIAP1 and cIAP2 are increased after labour in foetal membranes and myometrium and are essential for TNF-alpha-induced expression of pro-labour mediators. *Am J Reprod Immunol*. 2015;73:313–329.
13. Xu Y, Tarquini F, Romero R, et al. Peripheral CD300a+CD8+ T lymphocytes with a distinct cytotoxic molecular signature increase in pregnant women with chronic chorioamnionitis. *Am J Reprod Immunol*. 2012;67:184–197.
14. Kim CJ, Romero R, Chaemsaithong P, Kim JS. Chronic inflammation of the placenta: definition, classification, pathogenesis, and clinical significance. *Am J Obstet Gynecol*. 2015;213:S53–S69.

15. Gomez-Lopez N, Estrada-Gutierrez G, Jimenez-Zamudio L, Vega-Sanchez R, Vadillo-Ortega F. Fetal membranes exhibit selective leukocyte chemotaxic activity during human labor. *J Reprod Immunol*. 2009;80:122–131.
16. Gomez-Lopez N, Vadillo-Perez L, Hernandez-Carbajal A, Godines-Enriquez M, Olson DM, Vadillo-Ortega F. Specific inflammatory microenvironments in the zones of the fetal membranes at term delivery. *Am J Obstet Gynecol*. 2011;205:e215–e224.
17. Gomez-Lopez N, Vega-Sanchez R, Castillo-Castrejon M, Romero R, Cubeiro-Arreola K, Vadillo-Ortega F. Evidence for a role for the adaptive immune response in human term parturition. *Am J Reprod Immunol*. 2013;69:212–230.
18. Quinn KH, Parast MM. Decidual regulatory T cells in placental pathology and pregnancy complications. *Am J Reprod Immunol*. 2013;69:533–538.
19. Reinherz EL, Schlossman SF. The differentiation and function of human T lymphocytes. *Cell*. 1980;19:821–827.
20. Engleman EG, Benike CJ, Grumet FC, Evans RL. Activation of human T lymphocyte subsets: helper and suppressor/cytotoxic T cells recognize and respond to distinct histocompatibility antigens. *J Immunol*. 1981;127:2124–2129.
21. Biddison WE, Rao PE, Talle MA, Goldstein G, Shaw S. Possible involvement of the OKT4 molecule in T cell recognition of class II HLA antigens. Evidence from studies of cytotoxic T lymphocytes specific for SB antigens. *J Exp Med*. 1982;156:1065–1076.
22. Krensky AM, Reiss CS, Mier JW, Strominger JL, Burakoff SJ. Long-term human cytolytic T-cell lines allospecific for HLA-DR6 antigen are OKT4+. *Proc Natl Acad Sci USA*. 1982;79:2365–2369.
23. Meuer SC, Schlossman SF, Reinherz EL. Clonal analysis of human cytotoxic T lymphocytes: T4+ and T8+ effector T cells recognize products of different major histocompatibility complex regions. *Proc Natl Acad Sci USA*. 1982;79:4395–4399.
24. Burnet FM. *The clonal selection theory of acquired immunity Nashville*. Nashville, TN: Vanderbilt University Press; 1959.
25. Owen RD. Immunogenetic consequences of vascular anastomoses between bovine twins. *Science*. 1945;102:400–401.
26. Billingham RE, Brent L, Medawar PB. Actively acquired tolerance of foreign cells. *Nature*. 1953;172:603–606.
27. Schumacher A, Zenclussen AC. Regulatory T cells: regulators of life. *Am J Reprod Immunol*. 2014;72:158–170.
28. Abbas AK, Janeway CA Jr. Immunology: improving on nature in the twenty-first century. *Cell*. 2000;100:129–138.
29. Ellenhorn JD, Schreiber H, Bluestone JA. Mechanism of tumor rejection in anti-CD3 monoclonal antibody-treated mice. *J Immunol*. 1990;144:2840–2846.
30. Schneider E, Salaun V, Ben Amor A, Dy M. Hematopoietic changes induced by a single injection of anti-CD3 monoclonal antibody into normal mice. *Stem Cells*. 1997;15:154–160.
31. Meuer SC, Hodgdon JC, Hussey RE, Protentis JP, Schlossman SF, Reinherz EL. Antigen-like effects of monoclonal antibodies directed at receptors on human T cell clones. *J Exp Med*. 1983;158:988–993.
32. Leo O, Foo M, Sachs DH, Samelson LE, Bluestone JA. Identification of a monoclonal antibody specific for a murine T3 polypeptide. *Proc Natl Acad Sci USA*. 1987;84:1374–1378.
33. St Louis D, Romero R, Plazyo O, et al. Invariant NKT cell activation induces late preterm birth that is attenuated by rosiglitazone. *J Immunol*. 2016;196:1044–1059.
34. Furcron AE, Romero R, Mial TN, et al. Human chorionic gonadotropin has anti-inflammatory effects at the maternal-fetal interface and prevents endotoxin-induced preterm birth, but causes dystocia and fetal compromise in mice. *Biol Reprod*. 2016;94:136, 1–13.
35. Schulman H. The clinical implications of Doppler ultrasound analysis of the uterine and umbilical arteries. *Am J Obstet Gynecol*. 1987;156:889–893.
36. Electronic fetal heart rate monitoring: research guidelines for interpretation. The national institute of child health and human development research planning workshop. *J Obstet Gynecol Neonatal Nurs*. 1997;26:635–640.
37. Ferran C, Sheehan K, Dy M, et al. Cytokine-related syndrome following injection of anti-CD3 monoclonal antibody: further evidence for transient in vivo T cell activation. *Eur J Immunol*. 1990;20:509–515.
38. Jacques SM, Qureshi F. Chronic chorioamnionitis: a clinicopathologic and immunohistochemical study. *Hum Pathol*. 1998;29:1457–1461.
39. Kim JS, Romero R, Kim MR, et al. Involvement of Hofbauer cells and maternal T cells in villitis of unknown aetiology. *Histopathology*. 2008;52:457–464.
40. Kim MJ, Romero R, Kim CJ, et al. Villitis of unknown etiology is associated with a distinct pattern of chemokine up-regulation in the feto-maternal and placental compartments: implications for conjoint maternal allograft rejection and maternal anti-fetal graft-versus-host disease. *J Immunol*. 2009;182:3919–3927.
41. Shanmugasundaram U, Critchfield JW, Pannell J, et al. Phenotype and functionality of CD4+ and CD8+ T cells in the upper reproductive tract of healthy premenopausal women. *Am J Reprod Immunol*. 2014;71:95–108.
42. Trifonova RT, Lieberman J, van Baarle D. Distribution of immune cells in the human cervix and implications for HIV transmission. *Am J Reprod Immunol*. 2014;71:252–264.
43. Kim CJ, Romero R, Kusanovic JP, et al. The frequency, clinical significance, and pathological features of chronic chorioamnionitis: a lesion associated with spontaneous preterm birth. *Mod Pathol*. 2010;23:1000–1011.
44. Lee J, Kim JS, Park JW, et al. Chronic chorioamnionitis is the most common placental lesion in late preterm birth. *Placenta*. 2013;34:681–689.
45. Torrance HL, Bloemen MC, Mulder EJ, et al. Predictors of outcome at 2 years of age after early intrauterine growth restriction. *Ultrasound Obstet Gynecol*. 2010;36:171–177.
46. Lee J, Romero R, Dong Z, et al. Unexplained fetal death has a biological signature of maternal anti-fetal rejection: chronic chorioamnionitis and alloimmune anti-human leucocyte antigen antibodies. *Histopathology*. 2011;59:928–938.
47. Tracey KJ, Beutler B, Lowry SF, et al. Shock and tissue injury induced by recombinant human cachectin. *Science*. 1986;234:470–474.
48. Finkel MS, Oddis CV, Jacob TD, Watkins SC, Hattler BG, Simmons RL. Negative inotropic effects of cytokines on the heart mediated by nitric oxide. *Science*. 1992;257:387–389.

How to cite this article: Gomez-Lopez, N., Romero, R., Arenas-Hernandez, M., Ahn, H., Panaitescu, B., Vadillo-Ortega, F., Sanchez-Torres, C., Salisbury, K. S. and Hassan, S. S. (2016), Intraperitoneal injection of a monoclonal αCD3ε antibody induces preterm labor and birth. American Journal of Reproductive Immunology, 76: 386–390. doi: 10.1111/aji.12562