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

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Abstract

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 and 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

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DECLARATION OF INTEREST STATEMENT

The authors report no declarations of interest.

αCD3ε antibody decreased the fetal heart rate and increased the umbilical artery pulsatility index when compared to isotype controls.

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

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 that are critical for antigen specific immunity as well as 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: 1) CD4+ T helper (Th) cells, which respond to exogenous antigens presented through MHC class II signaling[19-23]; and 2) 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 which can promote T cell 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 (4-10μg) 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, since 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 via 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.

MATERIALS AND METHODS

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 h). Eight- to 12-week-old females were mated with males of proven fertility. Females were examined daily between 8:00 a.m. and 9:00 a.m. 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).

Intraperitoneal administration of a monoclonal αCD3ε antibody

Pregnant B6 mice were intraperitoneally injected with 10μg of a purified anti-mouse CD3ε (αCD3ε) (BD Biosciences, San Jose, CA, USA, Clone 145-2C11; n=12) dissolved in 200μL of sterile 1X phosphate-buffered saline (PBS) on 16.5 dpc. Controls were injected with 10μg of isotype (IgG1 κ Isotype; BD Biosciences, Clone A19-3; n=8) dissolved in 200μL 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.

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.

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/birthin 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 of 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. Fetal heart rate and umbilical artery pulsatility index (PI) were examined with the 55MHz 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 min after heating.

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 pulsatility index. A p value of 0.05 was considered statistically significant. When proportions are displayed, percentages and 95% confidence intervals are shown. Medians are shown with the interquartile range (IQR) and means are shown with the standard error of the mean (SEM).

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); $p<0.0001$; Figure 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); $p=0.002$; Figure 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); $p<0.0001$; Figure 1C].

Most of the dams injected with a monoclonal αCD3ε antibody delivered premature non-viable pups (Figure 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 (Figures 2A & 2B) prior to preterm labor/birth in mice injected with a monoclonal αCD3ε antibody or its time-matched isotype control. Fetuses from dams injected with a monoclonal αCD3ε antibody were viable, as a heartbeat was detected (Figure 2A). However, these fetuses were bradycardic when compared to controls [αCD3ε 104.32 bpm (SEM ± 4.11 bpm; n=88) vs. isotype 154.69 bpm (SEM ± 3.54 bpm; n=82); $p<0.0001$; Figure 2A]. Figure 2B shows how Doppler ultrasound is used to determine the blood flow through the umbilical artery. Fetuses from dams injected with a monoclonal αCD3ε antibody had an increased umbilical artery pulsatility index 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); $p=0.037$; Figure 2B]. Altogether, 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.

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 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 (CTL) 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 in the 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: 1) VUE is associated with an abnormal Doppler velocimetry of the umbilical artery[45]; and 2) 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, via a monoclonal αCD3ε antibody, induces fetal compromise and the premature expulsion of the semi-allograft fetus.

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REFERENCES

1. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008; 371:75–84. [PubMed: 18177778]

2. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. Science. 2014; 345:760–765. [PubMed: 25124429]
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. [PubMed: 17205421]
4. Romero R, Espinoza J, Kusanovic JP, Gotsch F, Hassan S, Erez O, Chaiworapongsa T, Mazor M. 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. [PubMed: 16839830]
6. Koga K, Cardenas I, Aldo P, Abrahams VM, Peng B, Fill S, Romero R, Mor G. Activation of TLR3 in the trophoblast is associated with preterm delivery. Am J Reprod Immunol. 2009; 61:196–212. [PubMed: 19239422]
7. Cardenas I, Means RE, Aldo P, Koga K, Lang SM, Booth CJ, Manzur A, Oyarzun E, Romero R, Mor G. Viral infection of the placenta leads to fetal inflammation and sensitization to bacterial products predisposing to preterm labor. J Immunol. 2010; 185:1248–1257. [PubMed: 20554966]
8. Cardenas I, Mulla MJ, Myrtolli K, Sfakianaki AK, Norwitz ER, Tadesse S, Guller S, Abrahams VM. Nod1 activation by bacterial iE-DAP induces maternal-fetal inflammation and preterm labor. J Immunol. 2011; 187:980–986. [PubMed: 21677137]
9. Romero R, Chaiworapongsa T, Alpay Savasan Z, Xu Y, Hussein Y, Dong Z, Kusanovic JP, Kim CJ, Hassan SS. 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. [PubMed: 21958433]
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. [PubMed: 24754320]
11. Kim YM, Romero R, Chaiworapongsa T, Kim GJ, Kim MR, Kuivaniemi H, Tromp G, Espinoza J, Bujold E, Abrahams VM, Mor G. 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. [PubMed: 15507964]
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. [PubMed: 25046208]
13. Xu Y, Tarquini F, Romero R, Kim CJ, Tarca AL, Bhatti G, Lee J, Sundell IB, Mittal P, Kusanovic JP, Hassan SS, Kim JS. 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. [PubMed: 22077960]
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:S53–69. [PubMed: 26428503]
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. [PubMed: 19406481]
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; 205:235, e215–224.
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. [PubMed: 23347265]
18. Quinn KH, Parast MM. Decidual regulatory T cells in placental pathology and pregnancy complications. Am J Reprod Immunol. 2013; 69:533–538. [PubMed: 23384284]
19. Reinherz EL, Schlossman SF. The differentiation and function of human T lymphocytes. Cell. 1980; 19:821–827. [PubMed: 6991122]
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. [PubMed: 6457863]

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. [PubMed: 6984061]
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 U S A. 1982; 79:2365–2369. [PubMed: 6980419]
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 U S A. 1982; 79:4395–4399. [PubMed: 6981813]
24. Burnet, FM. The clonal selection theory of acquired immunity Nashville. Vanderbilt University Press; Tennessee: 1959.
25. Owen RD. Immunogenetic Consequences of Vascular Anastomoses between Bovine Twins. Science. 1945; 102:400–401. [PubMed: 17755278]
26. Billingham RE, Brent L, Medawar PB. Actively acquired tolerance of foreign cells. Nature. 1953; 172:603–606. [PubMed: 13099277]
27. Schumacher A, Zenclussen AC. Regulatory T cells: regulators of life. Am J Reprod Immunol. 2014; 72:158–170. [PubMed: 24661545]
28. Abbas AK, Janeway CA Jr. Immunology: improving on nature in the twenty-first century. Cell. 2000; 100:129–138. [PubMed: 10647937]
29. Ellenhorn JD, Schreiber H, Bluestone JA. Mechanism of tumor rejection in anti-CD3 monoclonal antibody-treated mice. J Immunol. 1990; 144:2840–2846. [PubMed: 1969454]
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. [PubMed: 9090792]
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. [PubMed: 6604129]
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 U S A. 1987; 84:1374–1378. [PubMed: 2950524]
33. St Louis D, Romero R, Plazyo O, Arenas-Hernandez M, Panaitescu B, Xu Y, Milovic T, Xu Z, Bhatti G, Mi QS, Drewlo S, Tarca AL, Hassan SS, Gomez-Lopez N. Invariant NKT Cell Activation Induces Late Preterm Birth That Is Attenuated by Rosiglitazone. J Immunol. 2016; 196:1044–1059. [PubMed: 26740111]
34. Furcron AE, Romero R, Mial TN, Balancio A, Panaitescu B, Hassan SS, Sahi A, Nord C, Gomez-Lopez N. 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. [PubMed: 27146032]
35. Schulman H. The clinical implications of Doppler ultrasound analysis of the uterine and umbilical arteries. Am J Obstet Gynecol. 1987; 156:889–893. [PubMed: 2953241]
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, Schreiber R, Merite S, Landais P, Noel LH, Grau G, Bluestone J, Bach JF, 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. [PubMed: 2138557]
38. Jacques SM, Qureshi F. Chronic chorioamnionitis: a clinicopathologic and immunohistochemical study. Hum Pathol. 1998; 29:1457–1461. [PubMed: 9865833]
39. Kim JS, Romero R, Kim MR, Kim YM, Friel L, Espinoza J, Kim CJ. Involvement of Hofbauer cells and maternal T cells in villitis of unknown aetiology. Histopathology. 2008; 52:457–464. [PubMed: 18315598]
40. Kim MJ, Romero R, Kim CJ, Tarca AL, Chhauy S, LaJeunesse C, Lee DC, Draghici S, Gotsch F, Kusanovic JP, Hassan SS, Kim JS. 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. [PubMed: 19265171]

41. Shanmugasundaram U, Critchfield JW, Pannell J, Perry J, Giudice LC, Smith-McCune K, Greenblatt RM, Shacklett BL. 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. [PubMed: 24313954]
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. [PubMed: 24410939]
43. Kim CJ, Romero R, Kusanovic JP, Yoo W, Dong Z, Topping V, Gotsch F, Yoon BH, Chi JG, Kim JS. The frequency, clinical significance, and pathological features of chronic chorioamnionitis: a lesion associated with spontaneous preterm birth. Mod Pathol. 2010; 23:1000–1011. [PubMed: 20348884]
44. Lee J, Kim JS, Park JW, Park CW, Park JS, Jun JK, Yoon BH. Chronic chorioamnionitis is the most common placental lesion in late preterm birth. Placenta. 2013; 34:681–689. [PubMed: 23684379]
45. Torrance HL, Bloemen MC, Mulder EJ, Nikkels PG, Derks JB, de Vries LS, Visser GH. Predictors of outcome at 2 years of age after early intrauterine growth restriction. Ultrasound Obstet Gynecol. 2010; 36:171–177. [PubMed: 20217892]
46. Lee J, Romero R, Dong Z, Xu Y, Qureshi F, Jacques S, Yoo W, Chaiworapongsa T, Mittal P, Hassan SS, Kim CJ. 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. [PubMed: 22092404]
47. Tracey KJ, Beutler B, Lowry SF, Merryweather J, Wolpe S, Milsark IW, Hariri RJ, Fahey TJ 3rd, Zentella A, Albert JD, et al. Shock and tissue injury induced by recombinant human cachectin. Science. 1986; 234:470–474. [PubMed: 3764421]
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. [PubMed: 1631560]

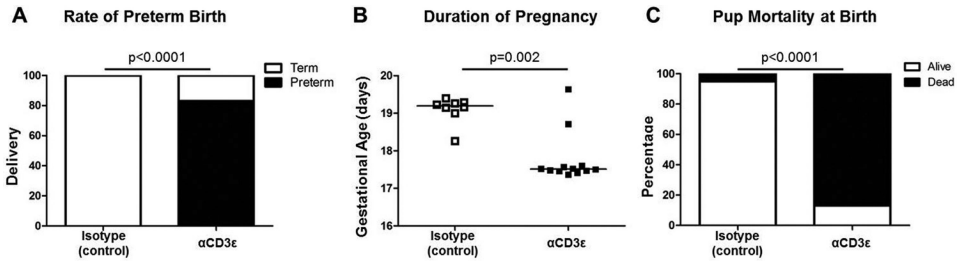

Figure 1.

Intraperitoneal injection of a monoclonal αCD3ε antibody. Pregnant B6 mice were intraperitoneally injected with a monoclonal αCD3ε antibody (10μg dissolved in 200μL of sterile PBS; n=12) on 16.5 days *post coitum* (dpc). Control mice were injected with an isotype (10μg dissolved in 200μL 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.

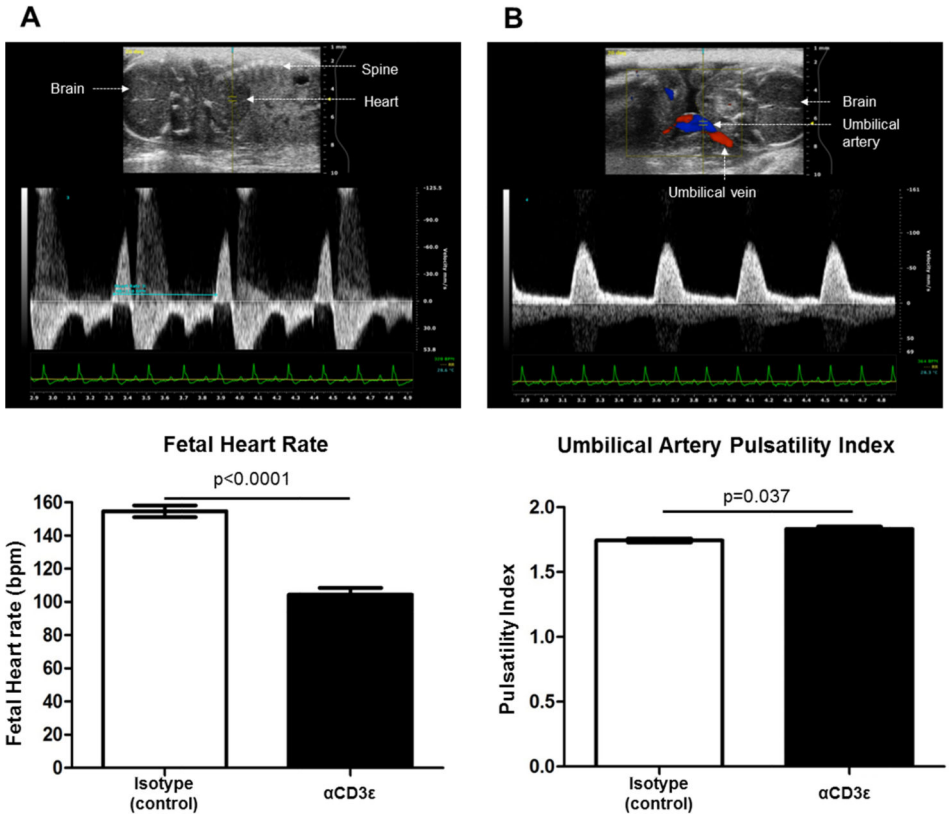

Figure 2.

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 (10μg dissolved in 200μL of sterile PBS; n=13) or time-matched isotype controls (10μg dissolved in 200μL of sterile PBS; n=12). Fetal heart rate (A) and umbilical artery pulsatility index (B) were recorded. Data are from 12-13 independent litters.