Title

Increased maternal T cell microchimerism in the allogeneic fetus during LPS-induced preterm labor in mice

Journal

Chimerism, 5(3-4)

ISSN

1938-1956

Authors

Wegorzewska, Marta
Le, Tom
Tang, Qizhi
et al.

Publication Date

2014-10-02

DOI

10.1080/19381956.2014.1002703

Peer reviewed

RESEARCH PAPER

Chimerism 5:3-4, 68–74; July–December 2014; © 2014 Taylor & Francis Group, LLC

Increased maternal T cell microchimerism in the allogeneic fetus during LPS-induced preterm labor in mice

Marta Wegorzewska[1,2], Tom Le[1,2], Qizhi Tang[2], and Tippi C MacKenzie[1,2,*]

[1]Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research; San Francisco, CA USA; [2]The Department of Surgery; University of California; San Francisco, CA, USA

Keywords: LPS-induced preterm labor, maternal-fetal cellular trafficking, maternal microchimerism, preterm labor

Abbreviations: PTL, preterm labor; MMc, maternal microchimerism; LPS, lipopolysaccharide; Tregs, regulatory T cells.

Fetal surgery is a promising strategy to treat fetuses with severe congenital abnormalities but its clinical applications are often limited by preterm labor. In normal pregnancy, multiple mechanisms protect the semi-allogeneic fetus from attack by maternal T cells. Maternal microchimerism (the presence of maternal cells in the fetus) has been suggested to be one mechanism of maternal-fetal tolerance in that it exposes the fetus to non-inherited maternal antigens and leads to the generation of fetal regulatory T cells that can suppress a maternal T cell response. Preterm labor may represent a breakdown of this robust tolerance network. We hypothesized that during inflammation-associated preterm labor, maternal leukocytes cross the maternal-fetal interface and enter the fetal circulation. Consistent with this hypothesis, we found that during preterm labor in mice, the percentage of maternal microchimerism in fetal blood increased and the frequency of fetuses with high levels of trafficking (greater than 0.5%) also increased. Finally, we showed that the maternal leukocytes trafficking into the fetus are primarily Gr-1$^+$ cells in both syngeneic and allogeneic pregnancy, while T cell trafficking into the fetus specifically increases during allogeneic pregnancies. Our results demonstrate that trafficking of maternal leukocytes during pregnancy is altered during preterm labor. Such alterations may be clinically significant in affecting maternal-fetal tolerance.

Introduction

Preterm birth (defined as birth prior to 37 weeks) is the most important cause of neonatal mortality and childhood morbidity in the developed world.[1] Surviving babies face long-term health problems, including neurodevelopmental impairments such as cerebral palsy and other learning disabilities.[2] Preterm labor (PTL) is multifactorial, but the leading cause is inflammation and infection.[3]

Although multiple mechanisms protect the semi-allogeneic fetus from the maternal immune system during normal pregnancy,[4–11] it is not known whether PTL entails recognition and rejection of the fetus by the maternal immune response. Maternal and fetal cells routinely traffic into each other's circulation[12,13] and the resultant bidirectional microchimerism may promote maternal-fetal tolerance at baseline.[14] Maternal cells in the fetus (Maternal microchimerism, MMc) reportedly expose the fetus to non-inherited maternal antigens and lead to the generation of fetal regulatory T cells (Tregs) that can suppress a maternal T cell response.[14] In healthy adults, maternal leukocytes have been shown to persist as various immune cell types.[15] Maternal cells have also been identified in children with Type I diabetes.[16,17] Although cellular trafficking may be a component of maternal-fetal tolerance, it has also been suggested that fetal-to-maternal trafficking is altered during pregnancy complications. For example, high levels of fetal DNA and cells have been found in maternal plasma in patients with spontaneous PTL[18] and levels of trafficking into the fetus increase after fetal surgery.[19]

Our lab has investigated the trafficking of maternal leukocytes into the fetus after fetal intervention. We recently reported that maternal T cells traffic into the fetus after allogeneic in utero haematopoietic cell transplantation in mice and may prevent engraftment of transplanted cells.[20] Moreover, maternal T cells play a role in the selective demise of fetuses after fetal stem cell transplantation in mice.[21] We have also observed that patients undergoing fetal surgery have increased levels of maternal cells in fetal blood.[19] However, trafficking in a model of PTL that more closely resembles human inflammation-induced PTL has not been reported.

Here, we hypothesized that during inflammation-associated preterm labor, maternal leukocytes cross the maternal-fetal interface and enter the fetal circulation. To test this hypothesis, we examined maternal-fetal cellular trafficking using an established method of lipopolysaccharide (LPS)-induced PTL in mice.[15] We

*Correspondence to: Tippi C. MacKenzie; Email: Tippi.Mackenzie@ucsfmedctr.org
Submitted: 09/08/2014; Revised: 12/16/2014; Accepted: 12/18/2014
http://dx.doi.org/10.1080/19381956.2014.1002703

demonstrate that LPS injection into the mouse uterus leads to increased MMc in the fetal blood. Moreover, there is a specific increase in the number of maternal T cells found in the fetus in allogeneic but not syngeneic pregnancies. These results suggest that maternal T cell awareness of fetal antigens is increased during PTL.

Results

LPS injection into the pregnant uterus results in preterm labor

We tracked maternal leukocytes in fetal blood in syngeneic and allogeneic pregnancies in a classic mouse model of PTL, intrauterine LPS injection.[22] We first did a dose-response study for intrauterine LPS in syngeneic pregnancies, injecting doses ranging from 0.05 μg to 1.00 μg. Injection of 0.05 μg resulted in delivery after 64 ± 21 (mean ± SEM) hours, whereas while higher concentrations (0.075, 0.100, 0.500, 1.00) resulted in an earlier delivery (36 ± 12–40 ± 16 hours) (**Fig. 1**). Time of delivery after treatment with 0.05 μg of LPS did not differ between syngeneic and allogeneic pregnancies (64 ± 21 hours vs 66 ± 15 hours) (**Fig. 1**). Based on these results, for our next set of experiments, we injected 0.05 μg of LPS to examine trafficking in fetal blood 12 hours after treatment to ensure viable pups for harvesting blood to analyze maternal cell trafficking.

Comparison of 2 methods to quantify maternal-fetal cellular trafficking during preterm labor

We next compared 2 methods for quantifying maternal cells in fetuses (MMc) (**Fig. 2A and B**). One was our previously published "leukocyte method,"[20] in which CD45.2 mothers are bred to CD45.1 fathers such that the fetuses are $CD45.1^{+}/CD45.2^{+}$ and maternal leukocytes are identified by lack of CD45.1. The second was the "GFP method,"[23] in which $GFP^{+/-}$ mothers are bred to $GFP^{-/-}$ fathers such that maternal cells can be tracked in the $GFP^{-/-}$ pups. To directly compare the 2 methods, we bred $B6.CD45.2.GFP^{+/-}$ mothers to $B6.CD45.1.GFP^{-/-}$ fathers and identified maternal cells as $CD45.1^{-}$ leukocytes in the pups (which are all $CD45.1^{+/-}$) or as GFP^{+} in $GFP^{-/-}$ pups (half of the litter, **Fig. 2A and B**). We induced PTL by intrauterine LPS injection on E16.5 and examined MMc in the blood of the pups after 12 hours using both methods. We found that the levels of MMc were the same with each method ($P = 0.76$ by paired t-test), such that they could be used interchangeably in subsequent analyses. (**Fig. 2C**).

Enhanced maternal cellular trafficking during preterm labor at sites of highest blood flow

We next quantified MMc during intrauterine LPS-induced PTL in syngeneic and allogeneic pregnancies. As above, we induced PTL on E16.5 and quantified the percentage and lineage composition of maternal leukocytes in fetal blood after 12 hours. We found low but detectable levels of maternal leukocytes in uninjected fetuses (0.1 ± 0 .1%), with no increase in trafficking after intrauterine PBS injection (syngeneic PBS, 0.3 ± 0 .1%; allogeneic PBS, 0.5 ± 0 .2%) (**Fig. 3A**). However, during LPS-induced PTL, the percentage of maternal leukocytes increased strikingly in the fetus whether the pregnancy was syngeneic or allogeneic (syngeneic LPS, 2 ± 0 .7% vs uninjected, $P < 0.05$; allogeneic LPS, 2 ± 0 .6% vs uninjected, $P < 0.05$ by Kruskal-Wallis test with Dunn's post-hoc comparison) (**Fig. 3A**). When we specifically examined fetuses with high levels (>0 .5%) of MMc, we determined that the pups of allogeneic matings undergoing PTL were most likely to have high MMc compared to the other groups (allogeneic PTL, 64%; allogeneic PBS, 38%; syngeneic PTL, 47%; syngeneic PBS, 27%; uninjected, 5%) (**Fig. 3B**). In allogeneic matings, the percentage of pups with high MMc increased during PTL compared to PBS injection (64% vs 38%, $p = 0.03$ by chi square test) (**Fig. 3B**). In syngeneic matings, there was no difference in the percentage of pups with high MMc during PTL compared to PBS injection ($p = 0$ .09 by chi square test). We also compared allogeneic and syngeneic pregnancies that were treated with LPS and found no difference either in the average number of trafficking cells or in the percent of pups with high trafficking ($p = 0.16$ by chi square test).

We next analyzed the location of fetuses with high levels of trafficking and determined that in both syngeneic and allogeneic pregnancies treated with LPS, fetuses with the highest levels of MMc were at the tops of the uterine horns

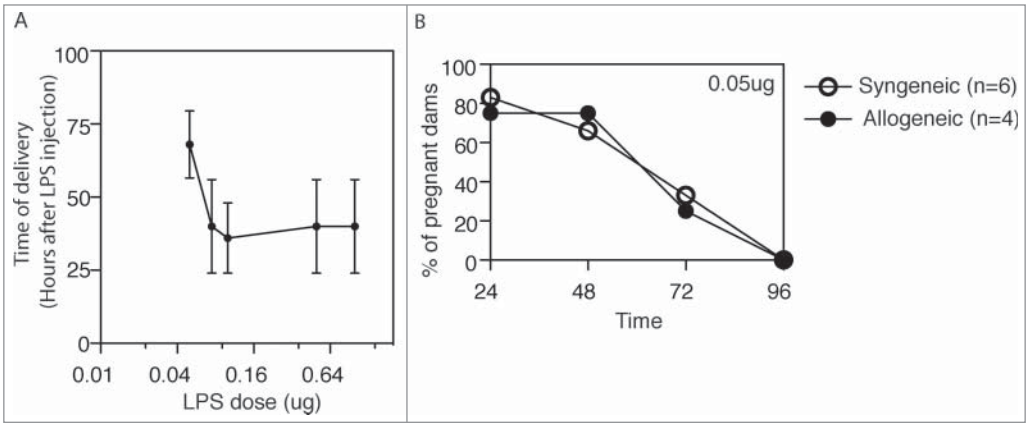

Figure 1. LPS injection into the pregnant uterus results in preterm delivery. (**A**) The time of delivery (in hours) after injecting various concentrations of lipopolysaccharide (LPS) into the uterus in syngeneic pregnancies (0.05 μg, n = 6; 0.075 μg, n = 3; 0.100 μg, n = 3; 0.500 μg, n = 3; 1.000 μg, n = 3). (**B**) The percentage of dams pregnant at 24, 48, 72 and 96 hours after injection of 0.5 μg of LPS into the uterus in syngeneic (n = 6) and allogeneic (n = 4) pregnancies.

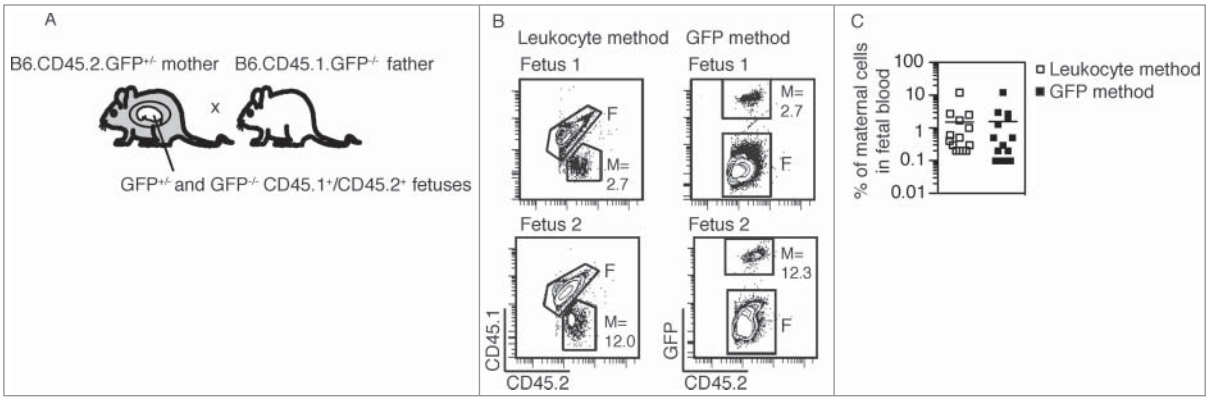

Figure 2. Comparison of 2 methods to track maternal-fetal cellular trafficking. (**A**) Breeding scheme in which $GFP^{+/-}$CD45.2 mothers are bred to $GFP^{-/-}$CD45.1 fathers such that resulting fetuses are either $GFP^{+/-}$ or $GFP^{-/-}$ and all fetuses are $CD45.1^{+}/CD45.2^{+}$. (**B**) Gating strategy to quantify maternal cells in fetal blood. In the leukocyte model, fetal cells are $CD45.1^{+}/CD45.2^{+}$ (gate F) and maternal cells are $CD45.2^{+}$ (gate M). In the GFP model, maternal cells are GFP^{+} and are identified only in $GFP^{-/-}$ pups (gate F). The percentage of maternal cells obtained using each method is demonstrated for 2 fetuses undergoing PTL. (**C**) Comparison of maternal cell chimerism levels using the leukocyte or GFP models (n = 15 fetuses). P = 0 .76 by paired t-test.

or near the cervix, and not necessarily near the site of LPS injection (**Figs. 3C–D**). These areas reportedly have the highest levels of blood flow,[24] suggesting a link between blood flow and trafficking.

Increase in maternal Gr-1 cells in fetal blood with a specific increase in T cells during preterm labor in allogeneic pregnancy

We further analyzed the lineage composition of the trafficking maternal leukocytes using markers for T cells (CD3), B cells (B220 or CD19) and granulocytes (Gr-1) (**Fig. 4A**). Most trafficking maternal leukocytes were $Gr\text{-}1^{+}$. When we compared the lineage distribution of maternal Gr-1, T, and B cells found in fetal blood to that found in maternal blood, the concentration of Gr-1 cells was higher in fetal blood, suggesting specific recruitment of these cells into the fetus rather than a non-specific breakdown of the maternal-fetal interface (**Fig. 4B**). Interestingly, the percentage of maternal T cells found in fetal blood during PTL was significantly higher in allogeneic pregnancies than that in syngeneic pregnancies ($13 \pm 3\%$ vs $4.1 \pm 1.1\%$, $P = 0.01$ by t-test) (**Fig. 4B**). Thus, although there were no differences in the overall leukocyte MMc between syngeneic and allogeneic matings, the lineage analysis indicated that T cell MMc was specifically increased in allogeneic matings. These results suggest that trafficking is one mechanism by which maternal T cells may be exposed to fetal antigens and develop increased awareness of the allogeneic fetus.

Discussion

This study demonstrates that during inflammation-associated PTL, there is increased maternal microchimerism of granulocytes in fetal blood, with a specific increase in T cells during allogeneic matings. In our experiments, we showed that during PTL, the percentage of MMc in fetal blood increased and the frequency of fetuses with high levels of trafficking (greater than 0.5%) also increased. We also showed that the maternal leukocytes trafficking into the fetus are primarily $Gr\text{-}1^{+}$ cells in both syngeneic and allogeneic pregnancy, and that T cell trafficking into the fetus is specifically higher during allogeneic pregnancies. The significant increase of maternal leukocytes in fetal blood compared to the composition in maternal blood suggests a preferential recruitment of cells or enhanced proliferation after trafficking into the fetus, rather than an anatomical breakdown of the placental barrier.

The route of entry of maternal leukocytes into the fetus remains a fascinating unanswered question. The most direct route of maternal cell trafficking may be through the maternal-fetal interface in the placental labyrinth. We also see a correlation between trafficking and maternal blood flow, in that fetuses with higher levels of MMc are located in the uterine horns or near the cervix, and suspect that T cells and $Gr1^{+}$ cells are specifically recruited to the inflamed uterus via the circulation and may become concentrated at the maternal-fetal interface. This interface is made up of 3 layers: the fetal mononuclear trophoblasts and a continuous double-layer of syncytiotrophoblasts.[25] In the vasculature, capture and adhesion of recruited cells occurs through the interaction between leukocytes and the endothelium, a process that is mediated by selectins. In the placenta, however, leukocytes do not come into contact with the endothelium, but rather with a mononuclear trophoblast layer and a continuous double-layer of syncytiotrophoblasts. In the murine maternal-fetal interface, there are increases in E-selectin expression on the trophoblasts that line maternal blood sinuses, and in VCAM-1 expression on maternal blood vessels during mid-gestation, and these changes correlate with neutrophil infiltration.[26] A study in humans has shown that L-selectin expression on trophoblasts could potentially enable leukocyte capture from the maternal circulation.[27] In addition, maternal T cells have been observed in fetal tissues of patients with villitis of unknown etiology,[28,29] with coordinate changes in placental chemokines,[30] suggesting that maternal leukocytes can penetrate the maternal-fetal interface in the placenta.

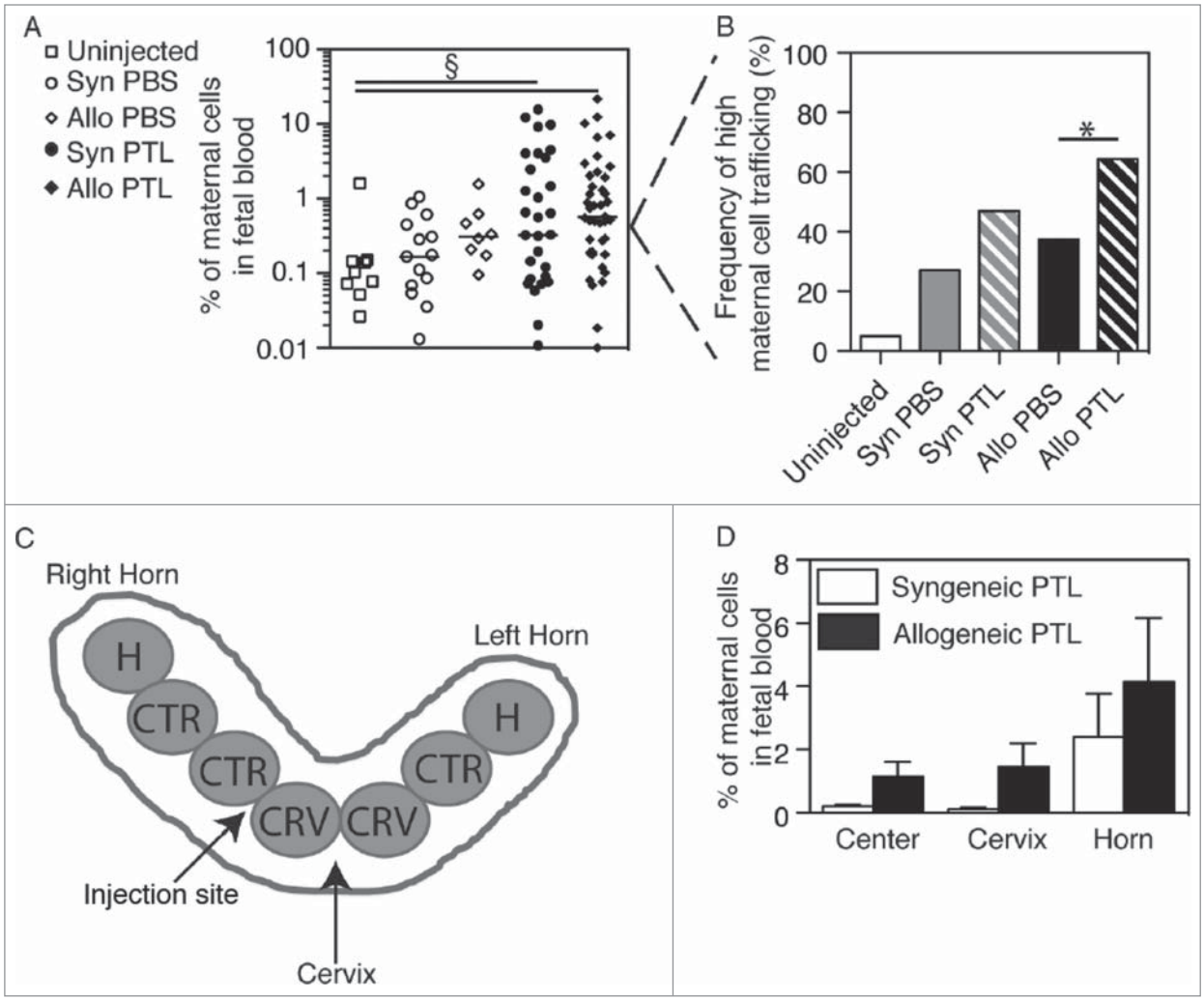

Figure 3. Increased presence of maternal leukocytes in fetal blood during preterm labor at sites of highest blood flow. (**A**) The percentage of trafficked maternal leukocytes among fetal leukocytes from uninjected (n = 19), PBS-injected (syngeneic (Syn PBS), n = 15 ; allogeneic (Allo PBS), n = 8), and LPS-injected fetuses (syngeneic (Syn PTL), n = 32 ; allogeneic (Allo PTL), n = 42). §P <0.05 by Kruskal-Wallis with Dunn's post-hoc comparison. (**B**) Frequency of pups with high levels (>0 .5%) of maternal microchimerism among all pups in the experimental group. *P<0.05 by Student's t-test. (**C**) Cartoon schematic defining specific locations within the mouse uterus. H = uterine horn; CTR = center; CRV = cervix. (**D**) The percentage of trafficked maternal leukocytes among fetal leukocytes from LPS-injected fetuses (syngeneic, n=32; allogeneic, n = 42) at various locations in the uterus (uterine horn, center or cervix).

Another route whereby maternal leukocytes may be gaining entry into fetal circulation is at the interface of the decidua (maternal) and trophoblast giant cells in the placenta. In normal pregnancy, epigenetic silencing of chemokine genes inhibits T cell migration into the decidua.[31] Alterations in chemokines during pregnancy complications are likely responsible for increased trafficking into the fetus. Previous studies in humans have shown that various chemokines produced by fetal membranes (CXCL8, CCL2, IP10, and MIP-1α) are associated with monocyte and T cell recruitment to the pregnant uterus during the onset of labor.[32,33] Selective recruitment of maternal leukocytes to the decidua has been described in mice [26] and humans[34] and it has been proposed that alterations in type of cells recruited to the decidua may result in pregnancy complications.[31]

One strength of this study is in our demonstration that 2 methods of quantifying MMc are equivalent. Our lineage analysis indicated that determination of lineage composition is a critical addition to quantifying overall levels of MMc. Thus, studies of human MMc should take into account the composition of trafficked maternal leukocytes in addition to the absolute levels of microchimerism. One weakness in our study is that we could not determine whether increased MMc is secondary to increased trafficking or secondary to increased proliferation of cells after entering the fetal circulation. Although we previously showed that adoptively transferred antigen-specific maternal T cells proliferate at the maternal-fetal interface,[21] there is an overall low level of trafficking in the fetus that precludes more detailed analysis of these cells. Our study also does not address whether maternal T cells contribute to the onset of preterm labor or whether

they simply proliferate in the inflammatory environment induced by LPS.

In conclusion, our study demonstrates that during inflammation-associated PTL, there is increased maternal microchimerism of granulocytes and T cells in fetal blood. We speculate that cellular trafficking after fetal intervention may influence maternal-fetal tolerance by facilitating maternal T cell activation through trafficking of maternal T cells and APC into the fetus and, possibly, release of fetal antigen into the maternal circulation. These results are consistent with our recent report that maternal T cells mediate resorption in a fetal intervention model.[21] Further studies are required to determine whether enhanced microchimerism has a functional role in the onset of LPS-induced preterm labor.

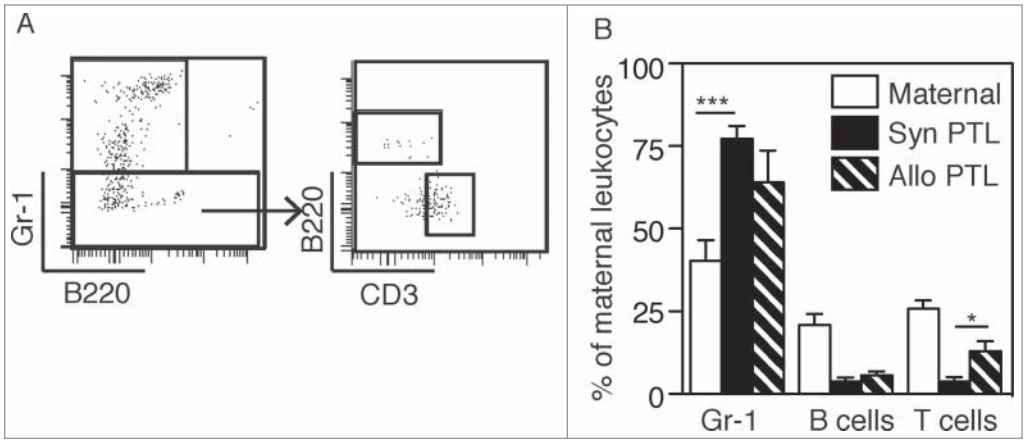

Figure 4. Increase in maternal Gr-1 cells in fetal blood with a specific increase in T cells during preterm labor in allogeneic pregnancy. (**A**) Gating strategy to phenotype trafficked maternal cells. Maternal leukocytes were separated into Gr-1$^+$ and Gr-1$^-$ gates. Gr-1$^-$ cells were divided into B220$^+$ (B cells) or CD3$^+$ (T cells). (**B**) Lineage analysis of trafficked maternal leukocytes during PTL shown as the percentage of all trafficked maternal leukocytes (e.g. trafficked Gr-1$^+$ cells/total trafficked maternal leukocytes) and compared to the lineage composition found in maternal blood. Syn: syngeneic mating; Allo: allogeneic mating. *P <0.05, ***P<0.001 by Student's t-test.

Materials and Methods

Reagents and antibodies

The following reagents were used: ACK Lysing Buffer (Lonza), dihydrochloride (DAPI, Invitrogen), Lipopolysaccharide from Salmonella abortus equi S-form (TLR-grade™) (LPS, Alexis Biochemicals). The following antibodies for flow cytometry were purchased from Becton Dickinson: CD3 (145–2C11), CD19 (1D3), CD45.1 (A20), CD45R/B220 (RA3–6B2); Ebioscience: CD45.1 (A20), CD45.2 (104); UCSF Hybridoma Core: Gr-1 (RB6–8C5), Fc receptor (2.4G2).

Mice

The inbred strains, BALB/c, C57BL/6.CD45.2 (B6), and C57BL/6.CD45.1 (CD45.1) were obtained from either NCI or Jackson Laboratories. B6.uGFP transgenic mice (strain 004353) were obtained from Jackson Laboratories. BALB/c.actin-GFP were a gift from Abul Abbas (University of CA, San Francisco, USA). All mice were bred and maintained in a specific pathogen-free facility at UCSF. All mouse experiments were performed according to the UCSF Institutional Animal Care and Use Committee approved protocol.

LPS-induced preterm labor

BALB/c and B6 mice were bred to create syngeneic and allogeneic pregnancies. On E16.5, a laparotomy was performed and 0.05 μg of LPS (or PBS) was injected into the uterus between the first and second fetus of the right horn to induce PTL.[22] Fetuses were harvested twelve hours later. The dose of LPS (0.05 μg, 0.075 μg, 0.100 μg, 0.500 μg, 1.000 μg) was titrated to ensure that it is a dose high enough to induce preterm delivery, but pups were harvested prior to delivery so that viable blood could be analyzed.

Tissue harvesting and flow cytometry

Fetuses were harvested 12 hours after maternal injection of LPS. Pups were washed twice in PBS prior to decapitation in heparinized HBSS to minimize contamination with maternal blood. Maternal blood was collected from the maxillary vein. The samples were stained for flow cytometry after first lysing the red blood cells in ACK lysis buffer as previously described.[20] At least 1,000,000 events were collected during the flow cytometry to ensure accurate analysis of microchimeric cells. "High traffickers" were defined as fetuses showing greater than 0.5% maternal microchimerism in the peripheral blood as previously described.[20]

Statistics

Comparisons involving 2 groups were evaluated using either the Chi-square test (for changes in frequency) or Student's t test. Comparisons between 2 or more groups were evaluated using ANOVA with a Tukey's multiple comparison test for pairwise comparisons, or with a Kruskal-Wallis test with Dunn's post-hoc comparison (the latter when the data were not normally distributed). A p value of less than 0.05 was considered to be significant. Data represent means ± SEM (or medians, for non-normally distributed data).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We would like to thank the Tang and Abbas labs, and Drs. Susan Fisher and Mike McCune for helpful discussions.

Funding

This work was supported by The California Institute for Regenerative Medicine (TCM), NIH/NIAID K08 AI085042 (TCM), National Science Foundation (MW), The March of Dimes (TCM), and the Pathology & Imaging Core of the UCSF Liver Center (P30 DK026743). The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of CIRM or any other agency of the State of California.

Author Contributions

M.W., Q.T., and T.C.M. designed the research and analyzed the data. M.W. and T.L. performed the experiments. M.W. and T.C.M. wrote the paper.

References

1. McCormick MC. The contribution of low birth weight to infant mortality and childhood morbidity. N Eng J Med 1985; 312:82-90; PMID:3880598; http://dx.doi.org/10.1056/NEJM198501103120204
2. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet 2008; 371:75-84; PMID:18177778; http://dx.doi.org/10.1016/S0140-6736(08)60074-4
3. Romero R, Dey SK, Fisher SJ. Preterm labor: one syndrome, many causes. Science 2014; 345:760-5; PMID:25124429; http://dx.doi.org/10.1126/science.1251816
4. Aluvihare VR, Kallikourdis M, Betz AG. Regulatory T cells mediate maternal tolerance to the fetus. Nat Immunol 2004; 5:266-71; PMID:14758358; http://dx.doi.org/10.1038/ni1037
5. Blois SM, Ilarregui JM, Tometten M, Garcia M, Orsal AS, Cordo-Russo R, Toscano MA, Bianco GA, Kobelt P, Handjiski B, et al. A pivotal role for galectin-1 in fetomaternal tolerance. Nat Med 2007; 13:1450-7; PMID:18026113; http://dx.doi.org/10.1038/nm1680
6. Guleria I, Khosroshahi A, Ansari MJ, Habicht A, Azuma M, Yagita H, Noelle RJ, Coyle A, Mellor AL, Khoury SJ, et al. A critical role for the programmed death ligand 1 in fetomaternal tolerance. J Exp Med 2005; 202:231-7; PMID:16027236; http://dx.doi.org/10.1084/jem.20050019
7. Hunt JS, Vassmer D, Ferguson TA, Miller L. Fas ligand is positioned in mouse uterus and placenta to prevent trafficking of activated leukocytes between the mother and the conceptus. J Immunol 1997; 158:4122-8; PMID:9126971
8. Mellor AL, Sivakumar J, Chandler P, Smith K, Molina H, Mao D, Munn DH. Prevention of T cell-driven complement activation and inflammation by tryptophan catabolism during pregnancy. Nat Immunol 2001; 2:64-8; PMID:11135580; http://dx.doi.org/10.1038/83183
9. Munn DH, Zhou M, Attwood JT, Bondarev I, Conway SJ, Marshall B, Brown C, Mellor AL. Prevention of allogeneic fetal rejection by tryptophan catabolism. Science 1998; 281:1191-3; PMID:9712583; http://dx.doi.org/10.1126/science.281.5380.1191
10. Erlebacher A, Vencato D, Price KA, Zhang D, Glimcher LH. Constraints in antigen presentation severely restrict T cell recognition of the allogeneic fetus. J Clin Invest 2007; 117:1399-411; PMID:17446933; http://dx.doi.org/10.1172/JCI28214
11. Xu C, Mao D, Holers VM, Palanca B, Cheng AM, Molina H. A critical role for murine complement regulator crry in fetomaternal tolerance. Science 2000; 287:498-501; PMID:10642554; http://dx.doi.org/10.1126/science.287.5452.498
12. Bianchi DW, Zickwolf GK, Weil GJ, Sylvester S, DeMaria MA. Male fetal progenitor cells persist in maternal blood for as long as 27 years postpartum. Proc Natl Acad Sci U S A 1996; 93:705-8; PMID:8570620; http://dx.doi.org/10.1073/pnas.93.2.705
13. Maloney S, Smith A, Furst DE, Myerson D, Rupert K, Evans PC, Nelson JL. Microchimerism of maternal origin persists into adult life. J Clin Invest 1999; 104:41-7; PMID:10393697; http://dx.doi.org/10.1172/JCI6611
14. Mold JE, Michaelsson J, Burt TD, Muench MO, Beckerman KP, Busch MP, Lee TH, Nixon DF, McCune JM. Maternal alloantigens promote the development of tolerogenic fetal regulatory T cells in utero. Science 2008; 322:1562-5; PMID:19056990; http://dx.doi.org/10.1126/science.1164511
15. Loubiere LS, Lambert NC, Flinn LJ, Erickson TD, Yan Z, Guthrie KA, Vickers KT, Nelson JL. Maternal microchimerism in healthy adults in lymphocytes, monocyte/macrophages and NK cells. Lab Invest 2006; 86:1185-92; PMID:16969370
16. Nelson JL, Gillespie KM, Lambert NC, Stevens AM, Loubiere LS, Rutledge JC, Leisenring WM, Erickson TD, Yan Z, Mullarkey ME, et al. Maternal microchimerism in peripheral blood in type 1 diabetes and pancreatic islet beta cell microchimerism. Proc Natl Acad Sci U S A 2007; 104:1637-42; PMID:17244711; http://dx.doi.org/10.1073/pnas.0606169104
17. Gammill HS, Nelson JL. Naturally acquired microchimerism. Int J Dev Biol 2010; 54:531-43; PMID:19924635; http://dx.doi.org/10.1387/ijdb.082767hg
18. Farina A, LeShane ES, Romero R, Gomez R, Chaiworapongsa T, Rizzo N, Bianchi DW. High levels of fetal cell-free DNA in maternal serum: a risk factor for spontaneous preterm delivery. Am J Obstet Gynecol 2005; 193:421-5; PMID:16098864; http://dx.doi.org/10.1016/j.ajog.2004.12.023
19. Saadai P, Lee TH, Bautista G, Gonzales KD, Nijagal A, Busch MP, Kim CJ, Romero R, Lee H, Hirose S, et al. Alterations in maternal-fetal cellular trafficking after fetal surgery. J Pediatr Surg 2012; 47:1089-94; PMID:22703775; http://dx.doi.org/10.1016/j.jpedsurg.2012.03.012
20. Nijagal A, Wegorzewska M, Jarvis E, Le T, Tang Q, MacKenzie TC. Maternal T cells limit engraftment after in utero hematopoietic cell transplantation in mice. J Clin Invest 2011; 121:582-92; PMID:21245575; http://dx.doi.org/10.1172/JCI44907
21. Wegorzewska M, Nijagal A, Wong CM, Le T, Lescano N, Tang Q, Mackenzie TC. Fetal intervention increases maternal T cell awareness of the foreign conceptus and can lead to immune-mediated fetal demise. J Immunol 2014; 192 (4); 1938-45; PMID:NOT_FOUND; http://dx.doi.org/10.4049/jimmunol.1302403
22. Elovitz MA, Wang Z, Chien EK, Rychlik DF, Phillippe M. A new model for inflammation-induced preterm birth: the role of platelet-activating factor and Toll-like receptor-4. Am J Pathol 2003; 163:2103-11; PMID:14578208; http://dx.doi.org/10.1016/S0002-9440(10)63567-5
23. Zhou L, Yoshimura Y, Huang Y, Suzuki R, Yokoyama M, Okabe M, Shimamura M. Two independent pathways of maternal cell transmission to offspring: through placenta during pregnancy and by breast-feeding after birth. Immunology 2000; 101:570-80; PMID:11122462; http://dx.doi.org/10.1046/j.1365-2567.2000.00144.x
24. Even MD, Laughlin MH, Krause GF, vom Saal FS. Differences in blood flow to uterine segments and placentae in relation to sex, intrauterine location and side in pregnant rats. J Reprod Fertil 1994; 102:245-52; PMID:7799320; http://dx.doi.org/10.1530/jrf.0.1020245
25. Maltepe E, Bakardjiev AI, Fisher SJ. The placenta: transcriptional, epigenetic, and physiological integration during development. J Clin Invest 2010; 120:1016-25; PMID:20364099; http://dx.doi.org/10.1172/JCI41211
26. Kruse A, Martens N, Fernekorn U, Hallmann R, Butcher EC. Alterations in the expression of homing-associated molecules at the maternal/fetal interface during the course of pregnancy. Biol Reprod 2002; 66:333-45; PMID:11804946; http://dx.doi.org/10.1095/biolreprod66.2.333
27. Genbacev OD, Prakobphol A, Foulk RA, Krtolica AR, Ilic D, Singer MS, Yang ZQ, Kiessling LL, Rosen SD, Fisher SJ. Trophoblast L-selectin-mediated adhesion at the maternal-fetal interface. Science 2003; 299:405-8; PMID:12532021; http://dx.doi.org/10.1126/science.1079546
28. Redline RW, Patterson P. Villitis of unknown etiology is associated with major infiltration of fetal tissue by maternal inflammatory cells. Am J Pathol 1993; 143:473-9; PMID:8342596
29. Myerson D, Parkin RK, Benirschke K, Tschetter CN, Hyde SR. The pathogenesis of villitis of unknown etiology: analysis with a new conjoint immunohistochemistry-in situ hybridization procedure to identify specific maternal and fetal cells. Pediatr Develop Pathol 2006; 9:257-65; PMID:16944988; http://dx.doi.org/10.2350/08-05-0103.1
30. Kim MJ, Romero R, Kim CJ, Tarca AL, Chhauy S, LaJeunesse C, Lee DC, Draghici S, Gotsch F, Kusanovic JP, 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; 182:3919-27; PMID:19265171; http://dx.doi.org/10.4049/jimmunol.0803834
31. Nancy P, Tagliani E, Tay CS, Asp P, Levy DE, Erlebacher A. Chemokine gene silencing in decidual stromal cells limits T cell access to the maternal-fetal interface. Science 2012; 336:1317-21; PMID:22679098; http://dx.doi.org/10.1126/science.1220030
32. 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;

80:122-31; PMID:19406481; http://dx.doi.org/10.1016/j.jri.2009.01.002
33. Gomez-Lopez N, Laresgoiti-Servitje E, Olson DM, Estrada-Gutierrez G, Vadillo-Ortega F. The role of chemokines in term and premature rupture of the fetal membranes: a review. Biol Reprod 2010; 82:809-14; PMID:20089887; http://dx.doi.org/10.1095/biolreprod.109.080432
34. Red-Horse K, Drake PM, Gunn MD, Fisher SJ. Chemokine ligand and receptor expression in the pregnant uterus: reciprocal patterns in complementary cell subsets suggest functional roles. Am J Pathol 2001; 159:2199-213; PMID:11733370; http://dx.doi.org/10.1016/S0002-9440(10)63071-4