



Reply to Keelan and Payne: Microbiota-related pathways for preterm birth

We thank Keelan and Payne (1) for their interest in our work (2). Their letter emphasizes intrauterine infection as a potential mechanistic link between the vaginal microbiota and preterm birth. Although we share their view of ascending infection as a possible mechanism, we don't consider it to be the only possible one. For this and other reasons we discuss below, we are hesitant to adopt the view—which we believe would risk introducing investigator biases—that documented intra-amniotic infection is a necessary accompanying condition for establishing a role of the microbiota in preterm birth.

As we report in our paper (2) and Keelan and Payne highlight in their letter (1), no women in our study had clinical chorioamnionitis. Lack of chorioamnionitis, however, isn't proof of absence of intra-amniotic infection because these infections are frequently subclinical. Others showed that among women with preterm labor and intact membranes who had a positive amniotic fluid culture, only 12.5% exhibited clinical chorioamnionitis (3). Keelan and Payne (1) also note that we presented no data on intra-amniotic infection status. Unfortunately, such data were precluded by the unavailability of amniotic fluid from our study population, an increasingly common scenario in the current era of noninvasive fetal assessment.

Notwithstanding the above challenges in linking intrauterine infection to the microbiota, we concur with Keelan and Payne's (1) salient point that our findings may have implications for preterm births unrelated to invasive infection. It is possible, for example, that certain vaginal bacterial communities are associated with increased risk

for noninfectious but inflammation-related preterm birth, including the need for an iatrogenic preterm delivery. Indeed, inflammation has been proposed as a final common pathway in both term and preterm deliveries (4). Under this paradigm, the microbiota might impact the timing of delivery by modulating systemic host immune responses. The immunomodulatory effects of a given bacterial community type could vary based on host features (e.g., race, ethnicity, metabolic factors, environmental exposures), including as-yet unknown or unsuspected genetic factors. Microbiota-mediated immune system programming that occurs before pregnancy, and especially during a shortened interpartum period (e.g., 12 mo or less), might alter the duration of a subsequent pregnancy. The critical nature of immune system programming by the microbiota is supported by studies in the postnatal period, demonstrating the importance of equilibrium between a host and its microbiota (5).

Given the complexity of preterm birth, including multiple clinical phenotypes, it is unrealistic to expect any single study to answer overarching questions in a definitive manner. Although our study has notable strengths (e.g., sampling at a relatively dense timescale, inclusion of multiple body sites, and analysis of the postpartum period), like all studies, ours also has limitations. The number of subjects ($n = 49$) was modest, even if the number of samples (~4,000) and sequences (~70M) was relatively robust. Also, as we mention in the *Discussion* section of our paper (2), our study population contained few black women and was clinically heterogeneous. Despite these limitations,

we believe that our findings enhance our understanding of the microbiota during and after pregnancy, and inform future investigations.

Daniel B. DiGiulio^{a,b}, David K. Stevenson^c, Gary Shaw^c, Deirdre J. Lyell^d, and David A. Relman^{a,b,e,1}

^aDepartment of Medicine, Stanford University School of Medicine, Stanford, CA 94305;

^bMedical Service, Veterans Affairs Palo Alto Health Care System, Palo Alto, CA 94304;

^cDepartment of Pediatrics, Stanford University School of Medicine, Stanford, CA 94305;

^dDepartment of Obstetrics & Gynecology, Stanford University School of Medicine, Stanford, CA 94305; and

^eDepartment of Microbiology & Immunology, Stanford University School of Medicine, Stanford, CA 94305

1 Keelan JA, Payne MS (2015) Vaginal microbiota during pregnancy: Pathways of risk of preterm delivery in the absence of intrauterine infection? *Proc Natl Acad Sci USA* 112:E6414.

2 DiGiulio DB, et al. (2015) Temporal and spatial variation of the human microbiota during pregnancy. *Proc Natl Acad Sci USA* 112(35):11060–11065.

3 Romero R, et al. (2001) The role of infection in preterm labour and delivery. *Paediatr Perinat Epidemiol* 15(Suppl 2):41–56.

4 Goldenberg RL, Hauth JC, Andrews WW (2000) Intrauterine infection and preterm delivery. *N Engl J Med* 342(20):1500–1507.

5 Garrett WS, Gordon JI, Glimcher LH (2010) Homeostasis and inflammation in the intestine. *Cell* 140(6):859–870.

Author contributions: D.B.D., D.K.S., G.S., D.J.L., and D.A.R. wrote the paper.

The authors declare no conflict of interest.

¹To whom correspondence should be addressed. Email: relman@stanford.edu.