competent; they were capable of entangling and killing bacteria (Figure 1F). Consistent with TLR ligand stimulation, live bacteria-induced NETosis was also delayed in neonatal neutrophils when monitored over 3 hours (data not shown).

When viewed in combination, these findings demonstrate that neonatal neutrophils exhibit an intrinsic delay in TLR2/TLR4-mediated NET formation, but are capable of releasing functionally competent NETs. The underlying cellular mechanisms and the clinical implications of neonatal NETosis delay remain to be addressed in future studies.

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Response

Gestational age as a factor in neutrophil extracellular trap formation

We appreciate the opportunity to comment on the letter and observations by Marcos and coworkers, and we agree that the experiments that they report using neutrophils from 3 neonates confirm our findings at early time points of stimulation of neonatal neutrophils[1] and suggest an intrinsic delay in Toll-like receptor 2 (TLR2)– and TLR4–mediated neutrophil extracellular trap (NET) formation. Because bacterial killing by human neutrophils has time-dependent features,[2,3] a 2- to 3-hour delay in NET formation may contribute to uncontrolled bacterial replication that is sufficient to escape containment and killing of the microbes by these leukocytes and other innate immune effector mechanisms. In our studies, we did not examine NET formation at time points beyond 2 hours because our analysis of this response by neutrophils from healthy adults routinely demonstrated NET formation within 15 to 30 minutes after stimulation. Similarly, in the original report by Brinkmann et al, NET release was detected as early as 10 minutes after stimulation, depending on the concentration of agonist.[4]

We assume that the neutrophils studied by Marcos et al were from full-term neonates, although their gestational ages are not stated. In our published[1] and unpublished studies we rarely ($< 5\%$ of the time) observed NET formation by neutrophils from full-term infants stimulated with lipopolysaccharide or platelet-activating factor for 2 hours. Parallel studies of neutrophils isolated from premature infants (< 30 weeks' gestation at birth) and stimulated under the same conditions never demonstrated NET formation[1] (C.C.Y., unpublished data, November 2006). We believe that impaired NET formation by neonatal neutrophils is due, at least in part, to a developmental delay in key regulatory mechanisms involved, and that NET formation varies in magnitude and efficiency based on gestational age. Delayed but present NET

formation by neutrophils from a small number of newborns, reported here by Marcos et al, is compatible with this interpretation. The time course of full development of NET-generating pathways and the capacity to form NETs by neutrophils of term infants in the period after birth are not yet characterized.

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