Comment on "Induced IL-17–Producing Invariant NKT Cells Require Activation in Presence of TGF-β and IL-1β"

Marie-Laure Michel and Maria C. Leite-de-Moraes

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Comment on “TLR9 Provokes Inflammation in Response to Fetal DNA: Mechanism for Fetal Loss in Preterm Birth and Preeclampsia”

Scharfe-Nugent and colleagues (1) have suggested that the use of chloroquine may reduce the frequency of preterm births. The drug acts as a TLR9 inhibitor, reducing inflammation and the concentration of IL-6. Other TLR9 antagonists produce their effect by lowering the level of IFN-α (2), and it would be important to test whether a similar effect has occurred using chloroquine, because then the drug could be used in an attempt to prevent cerebral palsy in premature infants. IFN-α used therapeutically can cause spastic diplegia indistinguishable from the spastic diplegic form of cerebral palsy (3), which is most common in premature infants. Levels of IFN-α are raised in term infants with cerebral palsy (4). However, it appears that levels of this cytokine in preterm infants with cerebral palsy have only been measured in one study (5–7). Chloroquine may reduce the levels of IFN-α if they are found to be raised and may prevent spastic diplegia. As the drug has been used extensively to treat malaria there should be few safety concerns. When used therapeutically, IFN-α causes spastic diplegia only in the first year of life. Injection of IFN-α into neonatal rats may provide an animal model of spastic diplegia (8), where the effect of chloroquine can be studied.

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References

Comment on “Induced IL-17–Producing Invariant NKT Cells Require Activation in Presence of TGF-β and IL-1β”

We read with interest the article by Monteiro et al. (1) reporting that noncommitted peripheral invariant NKT (iNKT) cells could be induced to produce IL-17 when activated in the presence of TGF-β and IL-1β irrespective of CD4 and NK1.1 expression. Our previous papers showed that IL-17–producing iNKT cells acquire their ability to secrete high levels of IL-17 in the thymus (2, 3). These iNKT17 cells are identified by their expression of ROR-γt and are in majority NK1.1+ and CD4+ (2, 3). To get inside the question raised by Graça’s team (1), we took advantage of ROR-γt-GFPTC mice to electronically sort NK1.1pos iNKT splenocytes into ROR-γt+ and ROR-γt- cells (Fig 1A). Using the same conditions described in Monteiro’s paper, we unexpectedly observed that NK1.1pos ROR-γtpos iNKT cells greatly proliferated (at least 10-fold) while the majority of the ROR-γt- counterpart failed to expand and survive (Fig. 1B, 1C). This could explain the high frequency of IL-17–producing cells observed by the authors (1) because even low numbers of ROR-γtpos iNKT cells in the beginning of their cultures could consistently proliferate and be the major iNKT cell subset at the end.

FIGURE 1. The NK1.1pos ROR-γtpos iNKT cell fraction expanded and produced higher levels of IL-17 when cultured in pro-Th17 conditions compared with its ROR-γt- counterpart. CD1d tetramer+ TCRβpos NK1.1pos iNKT splenocytes from ROR-γt-GFPTC C57BL/6 mice (A) were electronically sorted into NK1.1pos ROR-γt+ (B) or NK1.1pos ROR-γt- (C) cells and further cultured with plate-bound anti-CD3 and anti-CD28 mAb in the presence of IL-1β, IL-6, anti–IFN-γ, and TGF-β (all from R&D Systems). Four days later, cells were further stimulated for 4 h with PMA + ionomycin and then analyzed for cell viability using Live/Dead (Life Technologies) staining (B, C, left panels). ROR-γt expression and IL-17 production were then assessed among live cells (B, C, right panels). Representative dot plots and histograms (three distinct experiments using a pool of 2–5 mice) are shown. The percentage of the respective subsets is shown.
We cannot completely exclude the possibility that a small fraction of ROR-γt-negative iNKT splenocytes could acquire ROR-γt expression and become IL-17 producers in periphery upon pro-Th17 conditions (Fig 1B), but, consistent with our previous report (3), our present findings indicate that this is not a general rule for peripheral iNKT cells.

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References


Response to Comment on “Induced IL-17–Producing Invariant NKT Cells Require Activation in Presence of TGF-β and IL-1β”

The main conclusion of our manuscript was that uncommitted peripheral iNKT (iNKT) cells can acquire IL-17 secretion upon activation in the right microenvironment (1), in addition to the existence of a thymic-committed population of IL-17 secreting iNKT cells as previously reported (2). Michel and Leite de Moraes revisited our findings using ROR-γt-GFP reporter mice that were not available in our laboratory when we first performed our study. In their Letter to the Editor, it is claimed that the majority of IL-17γt iNKT cells observed following stimulation under IL-17–promoting conditions are derived from expansion of pre-existent ROR-γt-GFP precursors. However, given their low frequency, an exceptional proliferation rate would be necessary for those rare precursors to dominate the culture after days. To directly address this issue, we FAC-sorted equivalent populations of iNKT cells from ROR-γt-GFP reporter mice (Fig. 1A). We then cultured ROR-γt-GFP+ cells with unfractioned NK1.1γt iNKT cells from Thy1.1 mice at a 1:1 ratio (Fig. 1B, left column). These cultures confirmed that ROR-γt+ precursors have an advantage regarding survival and IL-17 production under IL-17–polarizing conditions (as we have suggested in our manuscript, where this proliferative advantage is observed even in the absence of added TGF-β). But the key experiment is the coculture of unfractioned NK1.1γt iNKT cells with a sorted population devoid of ROR-γt+ expressing precursors (Fig. 1B, right column). If the hypothesis that IL-17 secretion derives predominantly from precommitted ROR-γt+ precursors was correct, most IL-17γt iNKT cells should be Thy1.1γt—the only population with ROR-γt+ precursors. However, our data clearly show that the frequency of IL-17γt iNKT cells is similar in both Thy1.1γt and Thy1.2γt populations. These data conclusively show that uncommitted (ROR-γt−neg) iNKT cells, despite expanding and surviving less than ROR-γt+ precursors, make a significant contribution to the overall IL-17–secreting iNKT population, possibly due to their vast abundance in comparison with the rare ROR-γt+ precursors.

FIGURE 1. Uncommitted ROR-γt−neg iNKT cells can acquire an IL-17–producing phenotype. (A) Splenic iNKT cells were identified using a CD1d/PBS57 tetramer and TCRβGFP mice. The +GFP precursors. However, given their low frequency, an exceptional proliferation rate would be necessary for those rare precursors to dominate the culture after days. To directly address this issue, we FAC-sorted equivalent populations of iNKT cells from ROR-γt-GFP reporter mice (Fig. 1A). We then cultured ROR-γt-GFP+ cells

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The observation that ROR-γt+ precursors survive better than ROR-γtneg iNKT cells under IL-17-polarizing conditions is an original observation by Michel and Leite de Moraes, which we confirmed (Fig. 1B, top histograms). However, we reproducibly observed significant survival of ROR-γtneg iNKT cells (comparable to the survival of unfractioned Thy1.1+iNKT cells). We cannot offer a conclusive explanation for the extremely low survival of ROR-γtneg iNKT cells (∼1%) reported by Michel and Leite de Moraes. One possibility may be the use of a different culture medium. Unlike Michel and Leite de Moraes, we have used IMDM, and it has been reported that differences in IMDM and RPMI can significantly impact CD4+ Th17 polarization (3).

Overall, the use of ROR-γtGFP reporter mice allowed us to confirm that, in addition to “natural” thymic-derived iNKT17 cells, uncommitted iNKT cells can be peripherally induced to secrete IL-17.

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