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REVIEW

Involvement of decidual invariant NKT cells in inflammation-induced preterm delivery

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Invariant natural killer T (iNKT) cells are a distinct lineage of αβ T lymphocytes with the capacity to recognize glycolipid antigens in the context of the atypical major histocompatibility complex (MHC) class I molecule CD1d. In response to engagement of the T cell receptor (TCR), iNKT cells efficiently and rapidly produce a broad range of cytokines and several chemokines. iNKT cells participate in a variety of immune responses through cross-talk with many other innate and adaptive immune cells, including dendritic cells (DCs), natural killer (NK) cells, conventional $CD4^+$ T cells, $CD8^+$ T cells, B cells, neutrophils and regulatory T cells. Despite a relatively restrictive diversity in their TCR, these cells respond to vastly diverse microbial pathogens. The mechanisms underlying activation of iNKT cells at the maternal-fetal interface in inflammation-induced preterm delivery is not fully understood. In a recent study, we investigated which specific pathways were involved in decidual iNKT cell activation in a model for lipopolysaccharide (LPS)-stimulated preterm delivery. To do this, we employed an adoptive transfer system in combination with a diverse array of neutralizing antibodies (Abs) and inhibitors. We demonstrated that the activation of decidual iNKT cells requires TLR4-mediated nuclear factor-κB (NF-κB), mitogen-activated protein kinase (MAPK) p38 and extracellular signal-regulated kinase (ERK) pathways, the proinflammatory cytokines IL-12 and IL-18, and endogenous glycolipid antigens presented by CD1d. Our findings give new insights into the molecular mechanisms underlying iNKT cell activation during microbial infection as well as the role of iNKT cells in preterm delivery induced by inflammation. These findings underscore the promise that iNKT cell-based immunotherapies that target specific pathways utilized during iNKT cell activation could advance our ability to treat iNKT cell-associated inflammatory diseases, including preterm delivery.

Keywords: natural killer T cells; activation; preterm delivery; lipopolysaccharide; inflammation

Abbreviations: iNKT, Invariant natural killer T; MHC, major histocompatibility complex; TCR, T cell receptor; DCs, dendritic cells; NK, natural killer; LPS, lipopolysaccharide; NF-Kb, nuclear factor-κB; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; IFN, interferon; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; TNF, tumor necrosis factor; MIP-1α, macrophage inflammatory protein-1α; FasL, Fas ligand; WT, wild type; JNK, c-Jun N-terminal kinase; NB-DGJ, N-butyl-deoxygalactonojirimycin; β-GlcCe, β-D-glucopyranosylceramide

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Natural killer T (NKT) cells are a unique subset of T cells that have been described as one of the most potent modulators of innate and adaptive immunity [1]. While conventional T cells recognize peptide antigens presented by major histocompatibility complex (MHC) class I or II proteins, NKT cells specifically recognize glycolipid antigens presented by the MHC class I-like antigen presenting molecule CD1d [2]. CD1d-restricted NKT cells are subcategorized by their T cell receptor (TCR) repertoire and antigenicity profile into type I and type II NKT cells. To date, the best characterized NKT cells are type I or invariant NKT (iNKT) cells. The semi-invariant TCR expressed on these

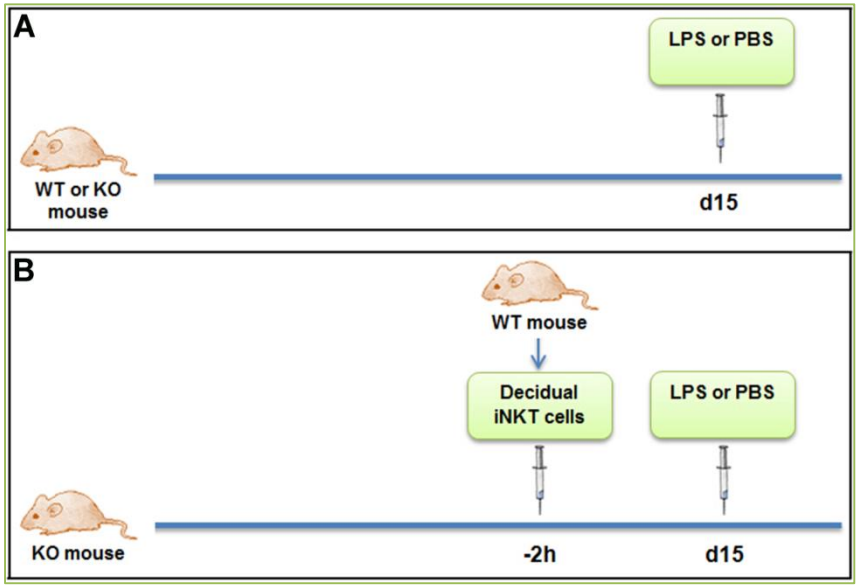

Figure 1. Protocol for the LPS-induced preterm delivery model and adoptive transfer of decidual iNKT cells. (A) Pregnant WT Jα18$^{+/+}$ mice or Jα18 KO mice were i.p. injected with LPS (100 μg/kg body weight) or PBS on day 15 of gestation. (B) Jα18$^{-/-}$ mice were injected with decidual iNKT cells from WT C57BL/6 mice 2 h prior to LPS or PBS injection on day 15 of gestation.

cells is comprised of an invariant TCR α chain (Vα14-Jα18 in mice and the homologous Vα24-Jα18 in humans) that pairs with a limited selection of TCR β chains (Vβ8.2, Vβ7 or Vβ2 in mice and Vβ11 in humans) [3]. It is notable that iNKT cells can very rapidly produce a wide array of cytokines in response to TCR engagement, including interferon (IFN)-γ, interleukin (IL)-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17, IL-21, granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor (TNF)-α, and several chemokines, including regulated upon activation normal T cells expressed and secreted (RANTES) and macrophage inflammatory protein-1α (MIP-1α) [4]. In a feed-forward fashion, activated iNKT cells can augment the immune response they initiated by stimulating other immune cells types, including dendritic cells (DCs) [5], natural killer (NK) cells [6], conventional CD4$^+$ T cells [7], CD8$^+$ T cells [8], B cells [9], neutrophils [10] and CD4$^+$CD25$^+$ regulatory T cells [11]. The ability of iNKT cells to utilize cytokine and chemokine-mediated mechanisms to modulate both innate and adaptive immunity highlights their central role in immune responsivity. iNKT cells also express high levels of granzyme B, perforin and Fas ligand (FasL), products consistent with direct and indirect involvement in cytolysis [12]. It is these unique properties that allow iNKT cells to regulate host defense responses and immune-related protection and pathology, including microbial immunity [13, 14], tumor immunity [15], autoimmunity [16, 17] and transplantation immunity [18].

Preterm delivery, defined as delivery occurring before 37 weeks' gestation, accounts for more than 12% of all births in the United States [19]. The prevalence of preterm delivery is increasing despite major advances in obstetric and neonatal care [20]. Preterm delivery is a major international challenge, accounting for at least 1 million perinatal and pediatric deaths, and is the single most significant contributor to perinatal and pediatric morbidity and mortality worldwide [21]. Infection is the leading cause of preterm delivery. In fact, infection of the gestational compartment is found in nearly 40% of cases of preterm delivery [22]. Maternal infection has been shown to stimulate immune responses through signaling events initiated by cell surface recognition molecules such as Toll-like receptors (TLRs) [23]. Ligand binding of TLR4 stimulates host inflammatory responses and is central to the pathogenesis of infection-associated preterm delivery [24].

In a recent study [25], we employed decidual iNKT cell adoptive transfer to elucidate the role of iNKT cells in lipopolysaccharide (LPS)-induced preterm delivery. There are two common ways to model LPS-induced preterm delivery in animals. They have important differences. One models localized intrauterine infection and involves anesthesia, laparotomy, and intrauterine injection of LPS [26]. The other models maternal systemic infection and involves intraperitoneal administration of LPS [27]. The localized inflammation model is thought to more closely mimic the clinical presentation of infection-related preterm delivery when compared to the systemic infection model and results in minimal maternal mortality and much more limited

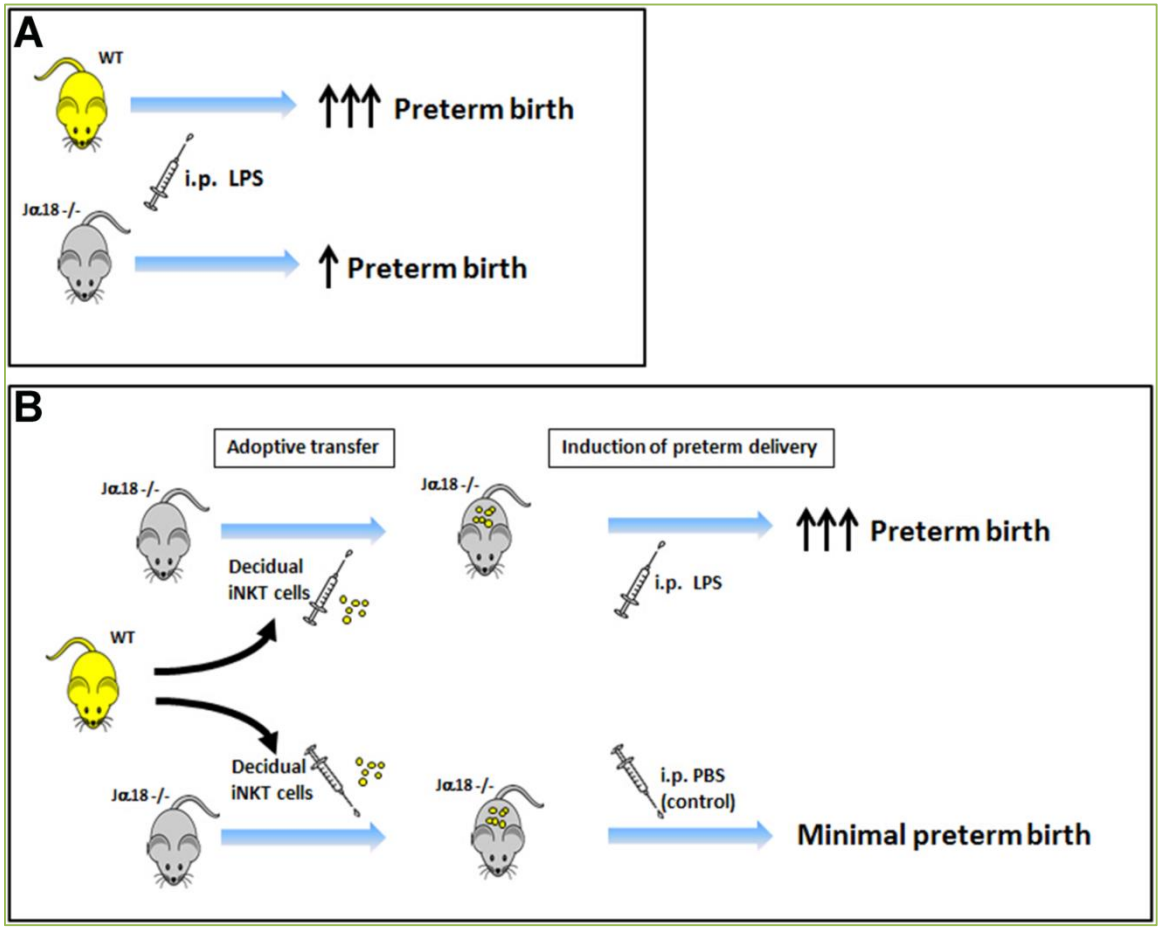

Figure 2. Effects of iNKT cells on LPS-induced preterm birth. (A) LPS treatment significantly increased preterm birth in WT mice, while deficiency of iNKT cells in Jα18 KO animals decreased LPS-induced preterm birth. (B) Adoptive transfer of decidual iNKT cells from WT mice into Jα18$^{-/-}$ mice significantly up-regulated preterm birth upon LPS stimulation, while adoptive transfer of decidual iNKT cells into Jα18$^{-/-}$ mice treated with PBS did not affect preterm birth.

maternal morbidity [26]. However, the involvement of surgical induction of the disease state is more complicated than mere i.p. injection, takes longer than the induction of the systemic inflammation model, and the required anesthesia and surgery does affect the dams in ways that may not be fully understood. Further, the sterile inflammation caused by the laparotomy may promote preterm delivery, although both effects may be mimicked in control animals that undergo sham operation. In contrast, while intraperitoneal injection of LPS is an effective and methodologically easy way to induce preterm delivery, maternal morbidity and even mortality are higher than that after surgical induction of local inflammation. That said, for our investigations, we successfully established a modified and less morbid murine model of preterm delivery that involved intraperitoneal injection of LPS (100 mg/kg body weight) on d15 of gestation. This i.p. LPS dosage and timing, while sufficient to induce preterm delivery, is accompanied by essentially no maternal mortality or detectable maternal morbidity.

The transgenic mice used in our study were Jα18 knockout (KO) mice. These animals are characterized by a deficiency in iNKT cells. Vα14 NKT cells express a single invariant TCR α chain encoded by the Vα14 and the Jα18 segments [3]. Conventional T cells do not express this TCR α chain. It is this unique expression of the invariant Vα14 TCR that allows specific deletion of the Jα18 gene to result in a selective loss of CD1d-dependent Vα14 NKT cells without affecting the other lymphoid lineages [28]. The development of the lymphoid organs in Jα18$^{-/-}$ mice is normal at a macroscopic level. Further, other than the complete loss of the Vα14 NKT cells, the numbers of local and circulating lymphocytes are very similar to those in wild type (WT) Jα18$^{+/+}$ mice [28]. Jα18$^{-/-}$ mice are known to be fertile and otherwise healthy [29].

The schematic protocol used in our investigations for the induction of preterm delivery and adoptive transfer of decidual iNKT cells is shown in Fig. 1. Our data showed that iNKT cell depletion significantly decreased preterm delivery in mice subjected to intraperitoneal administration of LPS.

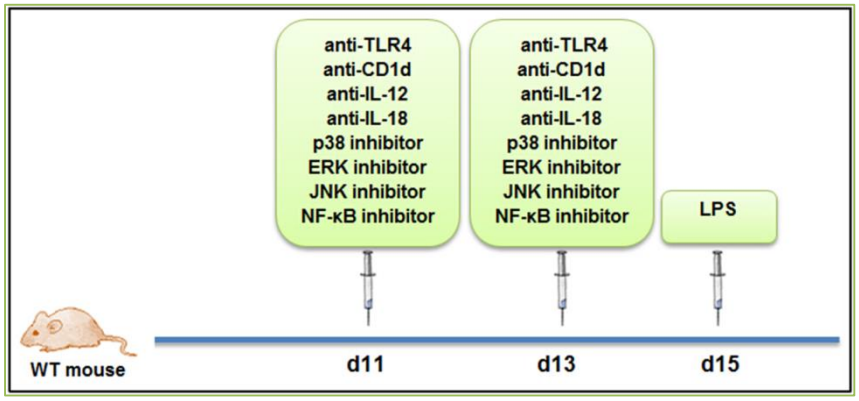

Figure 3. Protocol for the administration of neutralizing Abs and inhibitors. WT C57BL/6 mice were injected with neutralizing Abs against TLR4, CD1d, IL-12 and IL-18, or inhibitors blocking the activation of p38, ERK, JNK and NF-κB on day 11 and day 13 of gestation, and then injected with LPS on day 15 of gestation.

This suggests an important role for iNKT cells in LPS-induced preterm delivery. In addition, adoptive transfer of decidual iNKT cells into LPS-stimulated Jα18 KO mice from WT mice markedly increased preterm delivery, confirming the role of decidual iNKT cells in inflammatory preterm delivery (Fig. 2A). In contrast, transfer of donor decidual iNKT cells had no effect on the time to delivery in PBS-treated Jα18$^{-/-}$ mice. This supports the concept that exposure to these allogeneic cells will not, on its own, promote preterm delivery (Fig. 2B). In order to further explore the mechanisms underlying decidual iNKT cell-mediated preterm delivery, we assessed the percentages of decidual iNKT cells in recipient Jα18$^{-/-}$ mice. Adoptive transfer of decidual iNKT cells notably increased the percentage of decidual iNKT cells in LPS-exposed mice but not in PBS-treated Jα18$^{-/-}$ mice. This result suggested that LPS-induced systemic inflammation promotes iNKT cell migration to the recipient decidua. Further, donor decidual iNKT cell-mediated increases in preterm delivery may occur as the result of increases in the percentage of recipient decidual iNKT cells.

iNKT cells are powerful protective components in the host defense response to a considerable variety of microbial pathogens. Despite a markedly restricted TCR repertoire, these cells respond rapidly to a diverse array of microbial pathogens and display integral innate-like immune cell characteristics. iNKT cell activation appears to occur via two distinct pathways. One is a direct, microbial antigen-driven pathway. Some microbial glycolipids, such as glycosphingolipids (GSLs) from *Sphingomonas* [30, 31] and diacylglycerols from *Borrelia burgdorferi*, *Ehrlichia* and *Streptoccus pneumonia* [32, 33], can directly activate iNKT cells through the TCR. The other is TCR-independent and indirect. NKT cells can also respond to proinflammatory cytokines, such as IL-12, IL-18 or type I IFNs produced by antigen presenting cells upon TLR stimulation. This can be accomplished with [30, 34-36] or without [37-39] an accompanying weak TCR-mediated signal provided by recognition of CD1d-presented self-antigens.

The indirect pathway of iNKT cell activation was first described for the Gram-negative, LPS-positive microbe, *Salmonella typhimurium* [34]. Study of the pathogenesis of this infection revealed that IL-12 made by DCs in response to microbial products augments weak responses to CD1d-presented self-antigens and this, in turn, stimulates substantial IFN-γ secretion [34]. It has also been shown that *Salmonella* can trigger iNKT cells through the presentation of an endogenous lysosomal GSL, iGb3, by LPS-activated DCs [30]. In contrast, it has been reported that *Escherichia coli* LPS and *Salmonella abortus equi* LPS can activate iNKT cells through processes dependent on cytokines but independent of CD1d [37]. iNKT cell secretion of IFN-γ was not dependent on presentation of an endogenous antigen by CD1d in these studies. Instead, activation and IFN-γ secretion could be fully realized via culture in the presence of both IL-12 and IL-18.

Others have shown that iNKT cells can be activated in vitro or in vivo by an array of TLR agonists, including agonists of TLR2, TLR3, TLR5, TLR7, TLR8 and TLR9 [35, 36, 38, 39]. CpG oligodeoxynucleotides (ODN) and mouse cytomegalovirus (MCMV), which signal via TLR9, can stimulate iNKT cells to secrete IFN-γ but not IL-4. Also proposed is a requirement for DC-mediated type I IFN synthesis and de novo production of charged beta-linked GSLs in order for iNKT cell to be fully activated after ligation of TLR9 [35]. Still others have reported that this reaction only requires IL-12 and/or IFN-α/β secretion, but not CD1d expression [38, 39]. Furthermore, it has been demonstrated that iNKT cell IFN-γ production in response to in vitro stimulation or infection with a variety of bacterial species overwhelmingly depends on TLR-driven IL-12. Even

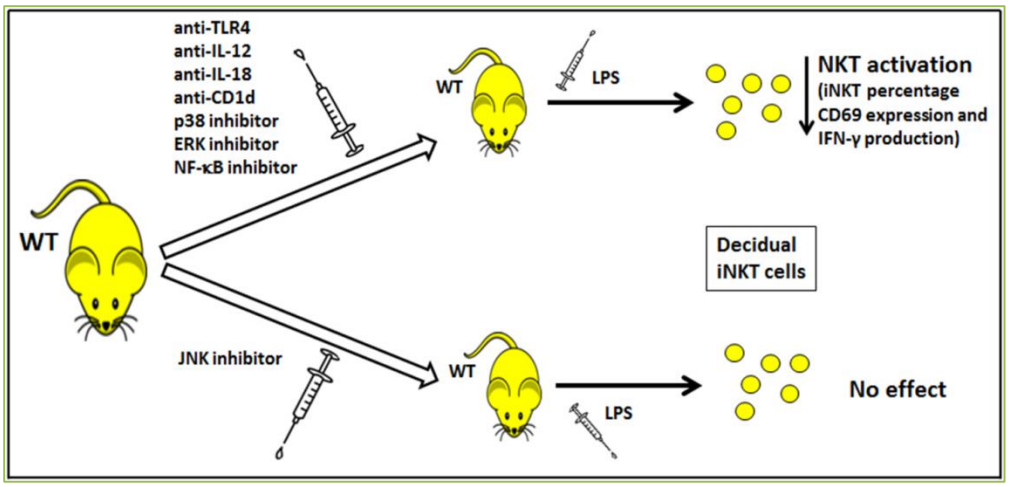

Figure 4. Effects of neutralizing Abs and inhibitors on LPS-induced preterm delivery. Neutralizing Abs against TLR4, IL-12, IL-18 and CD1d, or antagonists of p38, ERK and NF-κB markedly decreased LPS-mediated activation of decidual iNKT cells, while JNK inhibitor had no effect on inflammation-induced activation of decidual iNKT cells.

pathogens such as *Sphingomonas yanoikuyae* and *Streptococcus pneumoniae* that both require iNKT cells for optimal protection and express iNKT cell antigens can activate iNKT cells via mechanisms that mainly rely on IL-12. Together, these studies support the concept that iNKT cell activation during microbial infections is primarily driven by innate and cytokine-mediated signals and not by cognate microbial antigen [40].

In our study [25], we wanted to leverage the murine model of LPS-induced preterm delivery and a defined set of canonical neutralizing antibodies and inhibitors of specific steps involved in TLR4 signaling to better define specific underlying pathways involved in decidual iNKT cell activation. The antibodies and inhibitors were explicitly chosen to cover the full extent of the LPS signaling pathway, from initial ligand recognition and engagement of CD1d to the production and secretion the immune effector molecules, IL-12 and IL-18 (Fig. 3). Most of the inhibitory exposures utilized were demonstrated to markedly decrease readouts of LPS activity, including in vivo percentages of decidual iNKT cells, expression of CD69 and production of intracellular IFN-γ, as well as in vitro expression of CD69 on decidual iNKT cells and intracellular and extracellular secretion of IFN-γ in response to LPS (Fig. 4). Included among these proven inhibitory exposures were: 1) an inhibitor of mitogen-activated protein kinase (MAPK) p38, 2) an inhibitor of nuclear factor-κB (NF-κB) activation, 3) an inhibitor of extracellular signal-regulated kinase (ERK), 4) an anti-TLR4 neutralizing antibody, 5) an anti-IL-12 neutralizing antibody and 6) an anti-IL-18 neutralizing antibody (Fig. 4). Somewhat surprisingly, blockade of c-Jun N-terminal kinase (JNK) had no appreciable effect on the activation decidual iNKT cells (Fig. 4). Collectively, we were able to show that *Escherichia coli* LPS-induced decidual iNKT cell activation utilizes TLR4-mediated downstream ERK and NF-κB, p38 pathways and both IL-12- and IL-18-mediated signaling. Moreover, we proved that, while the IL-12 and IL-18 were each necessary for decidual iNKT cells activation, neither was sufficient. Further, IL-12 and IL-18, in combination, demonstrate synergism in their stimulatory effects on IFN-γ production by decidual iNKT cells.

It is known that CD1d-restricted iNKT cells are able to recognize a remarkably wide assortment of endogenous and exogenous lipid and glycolipid antigens [41]. In the thymus, CD1d-restricted recognition of endogenous glycolipid antigens is central to the appropriate selection and development of iNKT cells [42]. Moreover, the auto-reactivity of iNKT cells promotes a modified basal level of activation that enables suitable responses to subsequent co-stimulatory cytokines in the absence of the necessity for concurrent TCR stimulation [43]. The question of whether indirect activation of iNKT cells requires TCR-mediated signals generated after recognition of CD1d-presented endogenous glycolipids continues to be disputed. In our study, exposure to an antibody directed against CD1d reduced decidual iNKT cell percentages as well as their production of intracellular and extracellular IFN-γ and expression of surface CD69 upon LPS stimulation, all in the absence of exogenous antigens. These findings suggest that decidual iNKT cell activation is also contingent upon TCR stimulation by endogenous glycolipids presented by CD1d.

LPS has been reported to alter the synthesis of GSLs in a monocyte cell line [44]. This could increase presentation of glycolipid-derived self-antigens. Additionally, pretreatment

of DCs with *N*-butyl-deoxygalactonojirimycin (*N*B-DGJ), an inhibitor of β-D-glucopyranosylceramide (β-GlcCer) synthase, markedly reduces the ability of CpG ODN-stimulated DCs (or lipids extracted from these cells) to activate iNKT cells [35]. This is strong evidence that DCs produce charged β-GlcCer-derived GSLs in response to CpG ODN and that, together with type I IFNs, these GSLs can stimulate iNKT cells.

The TLR8 ligand R848 has been shown to enhance or alter the synthesis of GSLs and this contributes to the iNKT cell activation status [36, 43]. Moreover, β-GlcCer has been proposed as a potent iNKT cell self-antigen in mice and humans, and has been shown to contribute to iNKT cell activation in response to TLR agonists [45]. The identity of the subset of glycolipids directly involved in the physiologic stimulation of iNKT cells as opposed to those involved in iNKT cell activation in the presence of microbial infection remains undefined. The likelihood that endogenous glycolipids are involved but not yet identified provides yet another important aspiration for iNKT cell biologists.

In summary, our recent demonstration that TLR4-mediated pathways and IL-12 and IL-18 secretion in combination with autoantigenic TCR signaling are centrally involved in the activation of decidual iNKT cells by LPS adds to a growing body of literature suggesting an integral role for activated iNKT cells at the maternal-fetal interface in infection-associated preterm delivery [25, 46]. The indirect pathway of decidual iNKT cell activation may be particularly important in this respect, as it endows these cells with the ability to respond rapidly to a wide variety of microbes despite a limited TCR diversity, and thereby modulates inflammatory reactions at the maternal-fetal interface.

Acknowledgments

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Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Van Kaer L, Parekh VV, Wu L. Invariant natural killer T cells as sensors and managers of inflammation. Trends Immunol 2013; 34:50-58.
2. Kronenberg M, Kinjo Y. Innate-like recognition of microbes by invariant natural killer T cells. Curr Opin Immunol 2009; 21: 391-396.
3. Pellicci DG, Patel O, Kjer-Nielsen L, Pang SS, Sullivan LC, Kyparissoudis K, *et al*. Differential recognition of CD1d-α-galactosyl ceramide by the Vβ8.2 and Vβ7 semi-invariant NKT T-cell receptors. Immunity 2009; 31:47-59.
4. Coquet JM, Chakravarti S, Kyparissoudis K, McNab FW, Pitt LA, McKenzie BS, *et al*. Diverse cytokine production by NKT cell subsets and identification of an IL-17-producing CD4-NK1.1- NKT cell population. Proc Natl Acad Sci USA 2008; 105: 11287-11292.
5. Joyee AG, Uzonna J, Yang X. Invariant NKT cells preferentially modulate the function of $CD8\alpha^+$ dendritic cell subset in inducing type 1 immunity against infection. J Immunol 2010; 184: 2095-2106.
6. Moreno M, Molling JW, von Mensdorff-Pouilly S, Verheijen RH, von Blomberg BM, van den Eertwegh AJ, *et al*. In vitro expanded human invariant natural killer T-cells promote functional activity of natural killer cells. Clin Immunol 2008; 129:145-154.
7. Iwamura C, Shinoda K, Endo Y, Watanabe Y, Tumes DJ, Motohashi S, *et al*. Regulation of memory CD4 T-cell pool size and function by natural killer T cells in vivo. Proc Natl Acad Sci USA 2012; 109:16992-16997.
8. Reilly EC, Thompson EA, Aspeslagh S, Wands JR, Elewaut D, Brossay L. Activated iNKT cells promote memory $CD8^+$ T cell differentiation during viral infection. PLoS One 2012; 7:e37991.
9. Chung BK, Tsai K, Allan LL, Zheng DJ, Nie JC, Biggs CM, *et al*. Innate immune control of EBV-infected B cells by invariant natural killer T cells. Blood 2013; 122:2600-2608.
10. Wang H, Feng D, Park O, Yin S, Gao B. Invariant NKT cell activation induces neutrophil accumulation and hepatitis: opposite regulation by IL-4 and IFN-γ. Hepatology 2013; 58:1474-1485.
11. Venken K, Decruy T, Aspeslagh S, Van Calenbergh S, Lambrecht BN, Elewaut D. Bacterial CD1d-restricted glycolipids induce IL-10 production by human regulatory T cells upon cross-talk with invariant NKT cells. J Immunol 2013; 191:2174-2183.
12. Matsuda JL, Mallevaey T, Scott-Browne J, Gapin L. CD1d-restricted iNKT cells, the "Swiss-Army knife" of the immune system. Curr Opin Immunol 2008; 20:358-368.
13. Karmakar S, Bhaumik SK, Paul J, De T. TLR4 and NKT cell synergy in immunotherapy against visceral leishmaniasis. PLoS Pathog 2012; 8:e1002646.
14. Juno JA, Keynan Y, Fowke KR. Invariant NKT cells: regulation and function during viral infection. PLoS Pathog 2012; 8: e1002838.
15. Chu J, Deng Y, Benson DM, He S, Hughes T, Zhang J, *et al*. CS1-specific chimeric antigen receptor (CAR)-engineered natural killer cells enhance in vitro and in vivo antitumor activity against human multiple myeloma. Leukemia 2014; 28:917-927.
16. Parekh VV, Wu L, Olivares-Villagómez D, Wilson KT, Van Kaer L. Activated invariant NKT cells control central nervous system autoimmunity in a mechanism that involves myeloid-derived suppressor cells. J Immunol 2013; 190:1948-1960.
17. Oh SJ, Chung DH. Invariant NKT cells producing IL-4 or IL-10, but not IFN-γ, inhibit the Th1 response in experimental autoimmune encephalomyelitis, whereas none of these cells inhibits the Th17 response. J Immunol 2011; 186:6815-6821.

18. Knorr DA, Bachanova V, Verneris MR, Miller JS. Clinical utility of natural killer cells in cancer therapy and transplantation. Semin Immunol 2014; 26:161-172.

19. Shapiro-Mendoza CK, Lackritz EM. Epidemiology of late and moderate preterm birth. Semin Fetal Neonatal Med 2012; 17:120-125.

20. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet 2008; 371:75-84.

21. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, *et al*. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet 2012; 379:2162-2172.

22. Lamont RF. Infection in the prediction and antibiotics in the prevention of spontaneous preterm labour and preterm birth. BJOG 2003; 110:71-75.

23. Thaxton JE, Nevers TA, Sharma S. TLR-mediated preterm birth in response to pathogenic agents. Infect Dis Obstet Gynecol 2010; 2010: 378472.

24. Li L, Kang J, Lei W. Role of Toll-like receptor 4 in inflammation-induced preterm delivery. Mol Hum Reprod 2010; 16:267-272.

25. Li L, Yang J, Jiang Y, Tu J, Schust DJ. Activation of decidual invariant natural killer T cells promotes lipopolysaccharide-induced preterm birth. Mol Hum Reprod 2015; 21:369-381.

26. 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; 163:2103-2111.

27. Paintlia MK, Paintlia AS, Singh AK, Singh I. Attenuation of lipopolysaccharide-induced inflammatory response and phospholipids metabolism at the feto-maternal interface by N-acetyl cysteine. Pediatr Res 2008; 64:334-339.

28. Cui J, Shin T, Kawano T, Sato H, Kondo E, Toura I, *et al*. Requirement for Valpha14 NKT cells in IL-12-mediated rejection of tumors. Science 1997; 278:1623-1626.

29. Rogers L, Burchat S, Gage J, Hasu M, Thabet M, Willcox L, *et al*. Deficiency of invariant V alpha 14 natural killer T cells decreases atherosclerosis in LDL receptor null mice. Cardiovasc Res. 2008; 78:167-174.

30. Mattner J, Debord KL, Ismail N, Goff RD, Cantu C 3rd, Zhou D, *et al*. Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. Nature 2005; 434:525-529.

31. Kinjo Y, Wu D, Kim G, Xing GW, Poles MA, Ho DD, *et al*. Recognition of bacterial glycosphingolipids by natural killer T cells. Nature 2005; 434:520-525.

32. Kinjo Y, Tupin E, Wu D, Fujio M, Garcia-Navarro R, Benhnia MR, *et al*. Natural killer T cells recognize diacylglycerol antigens from pathogenic bacteria. Nat Immunol 2006; 7:978-986.

33. Kinjo Y, Illarionov P, Vela JL, Pei B, Girardi E, Li X, *et al*. Invariant natural killer T cells recognize glycolipids from pathogenic Gram-positive bacteria. Nat Immunol 2011; 12:966-974.

34. Brigl M, Bry L, Kent SC, Gumperz JE, Brenner MB. Mechanism of CD1d-restricted natural killer T cell activation during microbial infection. Nat Immunol 2003; 4:1230-1237.

35. Paget C, Mallevaey T, Speak AO, Torres D, Fontaine J, Sheehan KC, *et al*. Activation of invariant NKT cells by toll-like receptor 9-stimulated dendritic cells requires type I interferon and charged glycosphingolipids. Immunity 2007; 27:597-609.

36. Salio M, Speak AO, Shepherd D, Polzella P, Illarionov PA, Veerapen N, *et al*. Modulation of human natural killer T cell ligands on TLR-mediated antigen-presenting cell activation. Proc Natl Acad Sci USA 2007; 104:20490-20495.

37. Nagarajan NA, Kronenberg M. Invariant NKT cells amplify the innate immune response to lipopolysaccharide. J Immunol 2007; 178:2706-2713.

38. Tyznik AJ, Tupin E, Nagarajan NA, Her MJ, Benedict CA, Kronenberg M. Cutting edge: the mechanism of invariant NKT cell responses to viral danger signals. J Immunol 2008; 181: 4452-4456.

39. Wesley JD, Tessmer MS, Chaukos D, Brossay L. NK cell-like behavior of Valpha14i NK T cells during MCMV infection. PLoS Pathog 2008; 4:e1000106.

40. Brigl M, Tatituri RV, Watts GF, Bhowruth V, Leadbetter EA, Barton N, *et al*. Innate and cytokine-driven signals, rather than microbial antigens, dominate in natural killer T cell activation during microbial infection. J Exp Med 2011; 208:1163-1177.

41. Venkataswamy MM, Porcelli SA. Lipid and glycolipid antigens of CD1d-restricted natural killer T cells. Semin Immunol 2010; 22: 68-78.

42. Bendelac A, Savage PB, Teyton L. The biology of NKT cells. Annu Rev Immunol 2007; 25:297-336.

43. Wang X, Chen X, Rodenkirch L, Simonson W, Wernimont S, Ndonye RM, *et al*. Natural killer T-cell autoreactivity leads to a specialized activation state. Blood 2008; 112:4128-4138.

44. De Libero G, Moran AP, Gober HJ, Rossy E, Shamshiev A, Chelnokova O, *et al*. Bacterial infections promote T cell recognition of self-glycolipids. Immunity 2005; 22:763-772.

45. Brennan PJ, Tatituri RV, Brigl M, Kim EY, Tuli A, Sanderson JP, *et al*. Invariant natural killer T cells recognize lipid self antigen induced by microbial danger signals. Nat Immunol 2011; 12: 1202-1211.

46. Rinaldi SF, Rossi AG, Saunders PT, Norman JE. Immune cells and preterm labour: do invariant NKT cells hold the key? Mol Hum Reprod 2015; 21:309-312.