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NKT CELLS AT THE MATERNAL-FETAL INTERFACE

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Abstract

Establishment of the maternal-fetal interface is characterized by the influx of maternal NK cells, macrophages, and T cells into the decidua. Although a great deal has been learned about the function of NK cells in the decidua, comparatively little is known of decidual T cell function. NKT cells are an unusual T cell subset capable of producing both Th1-like and Th2-like cytokines. Unlike conventional αβ T cells that recognize peptides in the context of MHC molecules, NKT cells recognize glycolipids presented by the MHC class I-like molecule, CD1d. Recent reports have demonstrated that NKT cells and CD1d are present at the maternal-fetal interface. Moreover, activation of NKT cells can have dramatic effects on pregnancy. In this article, we will review basic aspects of NKT cell biology and summarize the recent literature on NKT cells at the maternal-fetal interface.

Keywords

NKT; CD1d; pregnancy; decidua; placenta; abortion

INTRODUCTION

Successful placentation is a dynamic process that, in humans and mice, is dependent on highly invasive fetal trophoblasts migrating through the decidualized endometrium where they degrade tissue and extracellular matrix through the production of a wide variety of proteinases and collagenases. Throughout their migration through the decidua, trophoblasts are in intimate contact with maternal decidual leukocytes, which comprise up to 40% of all cells in the decidua (Trundley and Moffett 2004). The local maternal immune response is characterized largely by cells of the innate arm, such as decidual NK cells and macrophages. Because they constitute the largest proportion of decidual lymphocytes (up to 70%), decidual NK cells have been the focus of the majority of research into decidual leukocyte function. In contrast, much less is known about the functions of decidual macrophages and T cells, which constitute up to 20% and 10% of the uterine leukocyte pool, respectively.

Recently, we and others reported the presence of NKT cells in the human and mouse decidua (Boyson et al. 2002; Ito et al. 2000; Tsuda et al. 2001). This small T cell subset has been demonstrated to modulate both innate and adaptive immune responses (Kronenberg and Gapin 2002) and, although the role of these cells in the decidua is unknown, experiments in the mouse have demonstrated that NKT stimulation dramatically impacts pregnancy, resulting in rapid pregnancy loss (Ito et al. 2000). Here, we review some basic aspects of NKT cell biology, summarize the literature to date regarding NKT cells at the maternal-fetal interface and finally, propose a model of possible decidual NKT cell function.

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NKT cells

NKT cells comprise a heterogeneous subset of αβ T cells characterized by the expression of both T cell and NK cell markers. Although the subset was originally identified in mice as NK1.1 + T cells, truncation of the term to NKT has resulted in a somewhat ambiguous term that encompasses a number of T cell subsets. As investigation into NKT cells has expanded across disciplines, this terminology has led to considerable confusion and has spurred attempts to develop a more standardized nomenclature (Godfrey et al. 2004)(Table I).

The most widely studied NKT cells are the classical NKT cells, also known as Type I, or iNKT cells. These NKT cells recognize glycolipids presented by the monomorphic class I MHC-like glycoprotein CD1d (Bendelac 1995a; Bendelac et al. 1995; Kawano et al. 1997). More specifically, classical NKT cells are characterized by their recognition of the prototypical CD1d ligand, the marine sponge glycosphingolipid alpha-galactosylceramide (αGalCer), which induces the rapid production of a wide variety of cytokines by the entire classical NKT cell subset (Kawano et al. 1997). Recognition is mediated through a semi-invariant T cell receptor (TCR), consisting of an invariant TCR α chain (Vα14-Jα18 in mouse or the homologous Vα24-Jα18 in human) paired preferentially with a variable (primarily $V\beta8^+$, $V\beta7^+$, or $V\beta2^+$ in the mouse, or Vβ11 in human) TCR β chain (Bendelac 1995b; Dellabona et al. 1994; Lantz and Bendelac 1994; Porcelli et al. 1996). Classical NKT cells are either CD4+CD8- or CD4-CD8- (DN), although CD8αα expression can be induced in human NKT cells after activation. Humans CD4+ and DN NKT cells are functionally distinct in that the DN population is characterized by a predominant Th1-like pattern of cytokine secretion, high levels of perforin expression, and high levels of NKG2D expression, while the CD4+ population secretes both Th1-like and Th2-like cytokines (Gumperz et al. 2002). In mice, however, these distinct differences are not as clear.

Most classical NKT cells are CD161 $(NK1.1)^+$. Identification of classical NKT cells using this marker alone, however, has proven problematic. First, only a few commonly used inbred mouse strains, such as C57BL/6, possess the CD161 alleles that encode the NK1.1 alloantigen recognized by the PK136 mAb (Carlyle et al. 2006). Second, accumulating data suggest the existence of $NK1.1^-$ classical NKT cells (McNab et al. 2007; Michel et al. 2007). Finally, it is becoming clear that non-classical NKT and CD1d–independent T cell subsets can also express NK1.1 (Behar and Cardell 2000b; Slifka et al. 2000). Likewise, in humans, most classical NKT cells express CD161, yet only a very small fraction of $CD161^+$ T cells are classical NKT (Lanier et al. 1994). It is not uncommon to find in the human literature the name NKT in reference to the $CD3^+CD56^+$ T cell population, though most data suggests that only a small fraction of this subset are classical NKT (Kim et al. 2002). Currently, the most accurate means with which to identify classical NKT cells is through the use of αGalCer-loaded CD1d multimers, some of which are available commercially (Proimmune, BD Biosciences) or through reagent resource programs (NIH tetramer facility).

The remainder of the CD1d–restricted T cell population is made up of non-classical (type II) NKT cells. These T cells differ from classical NKT cells in that they possess a diverse TCR repertoire and do not recognize αGalCer (Behar and Cardell 2000a; Cardell et al. 1995). Less is known about this T cell subset due to the lack of reagents with which to study them. Recent reports identifying sulfatide as a CD1d ligand that can stimulate non-classical NKT cells should pave the way for more thorough characterization of this subset (Zajonc et al. 2005). Interestingly, non-classical NKT cells appear to be quite different functionally from classical NKT cells, in that they appear to function as suppressor cells (Ambrosino et al. 2007; Halder et al. 2007; Terabe et al. 2005) and have been reported to suppress classical NKT cell function (Ambrosino et al. 2007). Thus, classical and non-classical NKT cells may each comprise part of an immunoregulatory network.

The remainder of the NKT cell population that co-express NK cell markers (e.g., CD3+CD56 + T cells in humans), and thus are "NKT-like," are not CD1d–restricted. This is a heterogeneous group of T cells which may include a number of diverse T cell subsets and they will therefore not be discussed here.

CD1d and glycolipids

Both type I and type II NKT cells recognize glycolipids presented by CD1d (Brigl and Brenner 2004). This is a monomorphic MHC class I-like molecule that is normally expressed on a variety of cells including B cells, dendritic cells, monocyte/macrophages, and epithelial cells (Kronenberg 2005). Although it is similar to classical class I MHC in that it is paired with β2m, CD1d differs in that it has a large, hydrophobic groove that accommodates the hydrophobic tails of its glycolipid ligands. The most widely used CD1d ligand is the marine sponge-derived glycosphingolipid αGalCer which activates all classical NKT cells (Kawano et al. 1997). However, although indispensable in its use as a tool with which to study classical NKT and CD1d biology, αGalCer is considered a non-physiological ligand since it cannot be found in vertebrates or microorganisms (Kinjo et al. 2005; Mattner et al. 2005; Sriram et al. 2005). Efforts by a number of laboratories to identify physiological exogenous ligands has led to the identification of bacterial cell wall glycolipids that stimulate classical NKT cells when presented by CD1d (Kinjo et al. 2005; Mattner et al. 2005; Sriram et al. 2005). In addition, one group has demonstrated that a melanoma-derived ligand, ganglioside GD3, is capable of being presented by CD1d to NKT cells (Wu et al. 2003).

NKT cells are unusual in that they show a propensity to recognize CD1d on cells in the absence of exogenous ligands. Thus, a number of laboratories have sought to identify endogenous CD1d ligands which has led to the identification of isoglobotrihexosylceramide (iGb3) as a mammalian endogenous ligand that stimulates both human and mouse NKT cells (Zhou et al. 2004), although there is controversy over its role in thymic selection (Porubsky et al. 2007). Therefore, NKT cells may recognize endogenous ligands produced in certain tissue microenvironments or during certain pathological states, or they may act in a more classical sense by recognizing pathogen glycolipids presented by CD1d.

NKT cells in the immune response

NKT cells influence a broad spectrum of immunological responses, including autoimmunity (Singh et al. 2001; Wilson et al. 1998), tolerance induction (Seino et al. 2001; Sonoda et al. 1999; Sonoda et al. 2001; Sonoda and Stein-Streilein 2002), tumor immunology (Cui et al. 1997; Kawano et al. 1998; Kawano et al. 1999; Smyth et al. 2000), and infectious disease (Hansen et al. 2003; Kinjo et al. 2005; Kumar et al. 2000; Mattner et al. 2005; Sriram et al. 2005). This diversity of function is due to the ability of NKT cells to rapidly produce a wide variety of cytokines such as IL-2, IL-4, IL-5, IL-10, IL-13, IL-17, IL-21, IFN-γ, and TNF-α, and GM-CSF (Kronenberg 2005). Production of both Th1-like as well as Th2-like cytokines may explain the paradoxical proinflammatory or tolerogenic ability of NKT cells exhibited in different contexts (Wilson and Delovitch 2003). Interestingly, the nature of the CD1d ligands themselves can bias NKT cells toward a Th1 or Th2 cytokine profile (Oki et al. 2004). OCH, a truncated version of αGalCer in which the sphingosine chain is shortened, elicits primarily a Th2 cytokine profile from classical NKT cells (Miyamoto et al. 2001). More recent work examining panels of synthetic glycolipids have underscored the ability of ligands to modulate the cytokine profiles of NKT cells. In addition to the obvious implications for therapeutic applications, these observations raise the possibility that different endogenous glycolipids may be associated with altered NKT cell function.

Direct and Indirect pathways for NKT cell activation

Exogenous CD1d glycolipid ligands, such as αGalCer, stimulate NKT cells after being endocytosed and loaded onto CD1d in APCs, a process that has been termed the direct pathway of NKT stimulation. Recently, it has been demonstrated that stimulation of APCs with TLR ligands such as LPS and CpG oligonucleotides induce the loading of endogenous mammalian glycolipid ligands on CD1d, that induce the activation of NKT cells. Since neither LPS nor CpG oligonucleotide is capable of stimulating NKT cells directly, this process has been termed the indirect pathway of NKT stimulation (Brigl et al. 2003; Paget et al. 2007). These intriguing observations suggest that NKT cells may be able to play a role in a broad spectrum of immunological responses to pathogens and that their response may not be limited only to pathogens that possess CD1d agonist glycolipid ligands. Indeed, evidence for this indirect pathway of NKT cell activation provides an explanation for the demonstration that NKT cells are critical in LPS-induced endotoxic shock (Dieli et al. 2000). Moreover, Nagarajan et al., demonstrated recently that NKT cells are involved in regulating TLR-induced macrophage TNF production (Nagarajan and Kronenberg 2007).

NKT cells, therefore, may play an early role in the development of the immune response and thus they are often referred to as a "bridge" between the innate and adaptive arms of the immune system. Apart from their ability to rapidly produce a wide variety of cytokines, activation of NKT cells results in the activation of a number of immune cell subsets (Fig. 1). NKT cell recognition of αGalCer on dendritic cells (DCs) leads to upregulation of CD40L on the NKT cell, which can then bind to CD40 on DCs and induce DC maturation and IL-12 secretion (Kitamura et al. 1999). In addition to DC activation, NKT cell activation leads to activation of B cells (Carnaud et al. 1999) as well as NK cells (Carnaud et al. 1999;Eberl and MacDonald 2000). The precise mechanism through which NK cell transactivation occurs is not known, although it is dependent on IL-12 and IFN-γ, and is mediated through DCs and/or macrophages (Bezbradica et al. 2005;Wesley et al. 2005a). Thus, NKT cells are emerging as potentially powerful modulators of both the innate and adaptive arms of the immune system.

NKT cells and CD1d at the maternal-fetal interface

NKT cells have been described in both the mouse and human decidua (Boyson et al. 2002; Ito et al. 2000; Tsuda et al. 2001). For the reasons discussed above, however, a variety of surrogate markers have been used to identify this population. Studies examining human decidual NKT cells are in general agreement that the classical NKT cell population is present at a frequency of approximately 0.5%, which is similar to what has been reported in the mouse (Boyson et al. 2002; Ito et al. 2000; Tsuda et al. 2001). Similarly, in both humans and mice, classical NKT cells appear to be highly activated and exhibit a Th1-like bias towards production of IFN-γ (Boyson et al. 2002; Ito et al. 2000). Although one report did not observe this Th1-like bias (Tsuda et al. 2001), the analysis was done on decidual $CD3^+V\alpha24^+$ cells, only a fraction of which are classical NKT cells (Boyson et al. 2002). As discussed earlier, there are few markers with which one can unambiguously identify the non-classical (type II) NKT population in either mice or humans. However, a recently published study describes that, in addition to classical NKT cells, the $CD3^+CD161^+$ decidual T cell population contains CD1d–restricted T cells that had diverse TCRs and exhibited a Th2-like cytokine profile, secreting IL-4 and IL-10 (Trapani et al. 1989). These characteristics are consistent with these cells being non-classical (Type II) NKT cells and suggest the presence of both types of NKT cells in the human decidua.

In contrast to the data in humans, there are somewhat conflicting reports regarding the composition of the murine decidual $NK1.1^+$ T cell population. One report suggested that $NK1.1^+$ T cells in the decidua expressed invariant TCR α chains and recognized αGalCer, and thus were classical NKT cells. It was noted, however, that decidual NKT cells preferentially expressed a Vβ7 TCR instead of Vβ8.2 that is more commonly observed in the spleen and liver

(Ito et al. 2000). These observations were supported by two other groups that detected invariant Vα14-Jα18 transcripts (Dang et al. 2000; Wang et al. 2002). However, it was also reported that while nearly all the decidual NK1.1$^+$ T cells are Vα14$^+$, there was preferential pairing with Vβ3 (Dang et al. 2000). In light of these data, it is intriguing that decidual NK1.1$^+$ T cells are dependent on β2m, but not CD1d (Dang and Heyborne 2001), indicating that they are restricted by an unknown β2m–associated protein. Even more interesting is that the restricting MHC class I molecule does not need to be expressed on maternal tissue for decidual NK1.1$^+$ T cell development. Instead, expression of β2m on paternally-derived tissue is sufficient for development of the decidual NK1.1+ T cell population, suggesting that this T cell subset develops extrathymically (Dang and Heyborne 2001). Clearly, a more thorough characterization of this T cell population is needed. Resolution of these issues will require characterization of the NK1.1$^+$ T cell population using αGalCer-loaded CD1d tetramers and functional assays.

Finally, a number of studies have documented in humans numerical and functional differences in the CD3$^+$CD56$^+$ NKT cell population. As indicated earlier, the identity of these cells is unknown and they have no obvious equivalent in the mouse. These cells are unlikely to be CD1d–restricted T cells since these markers have been demonstrated to be a poor predictor of CD1d–reactivity (Kim et al. 2002).

In addition to the presence of NKT cells at the human maternal-fetal interface, expression of CD1d has also been documented in early and in late gestation. Jenkinsson et al., first reported CD1d transcription in both trophoblast and choriocarcinoma cell lines (Jenkinson et al. 1999), which was followed by the demonstration that CD1d is expressed on both the villous and extravillous trophoblasts and that its expression increases as gestation progresses (Boyson et al. 2002; Shao et al. 2005). In addition to trophoblasts in anchoring villi, CD1d is expressed on trophoblasts that have invaded well into the maternal decidua (Boyson et al. 2002). Although it has never been formally demonstrated, this pattern of expression is consistent with the pattern observed for HLA-G and suggests that CD1d and HLA-G could be co-expressed on the same cells. In mice, the situation is again less clear than in humans. Although CD1d transcripts are clearly present in early gestation (day 6–8) decidual tissue (data not shown) and CD1d protein expression on decidual cell preparations has been reported (Dang et al. 2000), the cells which express CD1d have yet to be defined.

In addition to CD1d expression, the placenta possesses other features conducive to productive CD1d–NKT cell interactions. For example, it was recently demonstrated that a number of genes involved in sphingolipid metabolism were highly upregulated in the mouse decidua, and that disruption of sphingolipid metabolism caused pregnancy loss in mice (Mizugishi et al. 2007). In addition, prosaposin, an accessory molecule essential for glycosphingolipid loading onto CD1d (Kang and Cresswell 2004; Zhou et al. 2004) is known to be expressed at high levels in the decidua (Sun et al. 1994). Finally, although the profile of glycolipids in the placenta is still unclear, it is intriguing that ganglioside GD3, a recently described CD1d agonist ligand (Wu et al. 2003), has been demonstrated to be a predominant species in the rat placenta (Itonori et al. 1995).

NKT cells and immune-mediated pregnancy loss

There is a large body of literature in humans linking pregnancy loss with the presence of infection and pro-inflammatory cytokines (Buhimschi et al. 2005; Engel et al. 2005; Goepfert et al. 2001; Goldenberg et al. 2000; Lockwood and Kuczynski 1999; Romero et al. 2004). In light of the fact that recognition of bacterial cell wall glycolipids by NKT cells results in the production of large amounts of IFN-γ and TNF (Kinjo et al. 2005; Mattner et al. 2005; Sriram et al. 2005), it is interesting that activation of NKT cells with the agonist CD1d ligand αGalCer

rapidly induces pregnancy loss in C57BL/6J mice at all stages of gestation (Boyson et al. 2006; Ito et al. 2000).

The mechanisms through which αGalCer induces pregnancy loss are still unclear. It is ineffective in NKT- or CD1d–deficient mice, indicating that NKT cells are required and is dependent on TNF-α, IFN-γ, and perforin (Boyson et al. 2006; Ito et al. 2000). Recently, it was demonstrated that NKT cell activation induced preterm birth as well as early to mid-gestation pregnancy loss (Boyson et al. 2006). Surprisingly, key differences were observed in the mechanisms between early and mid-late gestation pregnancy loss, with perforin being required for early gestation pregnancy loss (up to day 8), but not during mid-late gestation (day 9 and after). Conversely, mid-late, but not early, gestation pregnancy loss was associated with strain-dependent variability in serum cytokine production. Significantly greater levels of a number of cytokines, including TNF, are produced in response to αGalCer in highly susceptible C57BL/6J mice, whereas these levels are lower in resistant strains such as BALB/cJ and C3H/HeJ (Boyson et al. 2006)(data not shown). Interestingly, these observations were similar to those seen in a model of pregnancy loss induced by CD40 cross-linking, which efficiently induced pregnancy loss early in gestation (up to day 8) but was ineffective at later stages of gestation (Erlebacher et al. 2004). Collectively, these observations suggest that there are distinct requirements for immune-mediated pregnancy loss at different stages of gestation.

Since NKT cells are known to activate a number of other leukocyte subsets, it is entirely possible that pregnancy loss due to their activation is only the first step in a cascade of events. Although Ito et al., speculated that NKT cells were directly involved in trophoblast cell death (Ito et al. 2000), NKT cells are present in relatively low numbers compared to uNK cells which share many effector functions with NKT cells. It is possible, therefore, that uNK cells contribute to NKT cell-mediated pregnancy loss. Mouse uNK cells, which are analogous to decidual $CD16^{-}CD56^{bright}$ NK cells, have recently been demonstrated to be critical in maternal decidual artery remodeling via their production of IFN-γ (Bilinski et al. 2008). Conversely, uNK cells have also been implicated in mouse models of pregnancy loss (Chaouat 1994; de Fougerolles and Baines 1987; Erlebacher et al. 2004; Murphy et al. 2005). With this in mind, it is interesting that NKT cell activation via αGalCer is known to result in spleen NK cell activation (Carnaud et al. 1999; Eberl and MacDonald 2000), a process known as “transactivation.” Through a mechanism that is still unclear, but which probably involves IFN-γ secretion by NKT cells and IL-12 secretion by DCs and/or macrophages (Bezbradica et al. 2005; Wesley et al. 2005b), NK cells are activated within hours of NKT cell activation, and begin to produce IFN-γ and increase their cytotoxic activity.

Thus, it is possible that NKT-mediated pregnancy loss may involve uNK cell transactivation. To test the hypothesis that an NKT-NK axis could function in the decidua, αGalCer was administered to pregnant mice on day 7.5 of gestation. Splenocytes and decidual leukocytes were isolated 5 hours later and IFN-γ production was assessed by intracellular staining. This preliminary analysis revealed that, similar to spleen NK cells, decidual uNK cells became activated and produced IFN-γ within hours of NKT cell activation (data not shown). Whether this mechanism is critical in mediating NKT-induced pregnancy loss, however, is still unknown. Also unclear is whether functional NKT-NK interactions take place in the absence of an agonist ligand such as αGalCer. However, the demonstration that NKT cell function is capable of modulating decidual uNK cell function, especially uNK cell IFN-γ production, argues that further examination of the NKT-NK axis at the maternal-fetal interface is warranted.

A role for NKT cells in LPS-induced pregnancy loss??

The induction of pregnancy loss by endotoxin (LPS) has been studied for at least sixty years (Zahl and Bjerknes 1943). It is a widely used model to study the effects of inflammation on pregnancy loss at all stages of gestation. As discussed above, there is a growing body of

evidence to suggest that NKT cells may play a critical role in many of the classic immune responses that occur after TLR cross-linking by LPS or CpG (Brigl et al. 2003; Paget et al. 2007). For example, serum TNF production as well as NK cell IFN-γ production in response to LPS are dramatically reduced in NKT-deficient mice (Nagarajan and Kronenberg 2007). A model has emerged in which NKT cells act to "license" innate immune cells such as macrophages or DCs to reach their full functional capacity. It is possible therefore, to devise a hypothesis (Fig. 2) in which NKT cells could play a role in immune-mediated pregnancy loss through direct recognition of bacterial cell wall glycolipids (direct pathway), or through recognition of endogenous agonist ligands loaded onto CD1d as a result of TLR ligation (indirect pathway).

CONCLUSIONS

Successful pregnancy requires the coordinate regulation of the innate and adaptive arms of the immune system. CD1d–restricted NKT cells are a novel subset of T cells with a demonstrated ability to modulate the function of both the innate and adaptive arms of the immune system. Especially interesting with regard to their possible function at the maternal-fetal interface is their ability to mediate both pro-inflammatory or tolerogenic immune responses in a context-dependent manner. Although we still know very little of their function at the maternal-fetal interface, their ability to dramatically impact pregnancy upon activation with an agonist ligand, together with the demonstration that they may be able to modulate decidual uNK cell function, suggests that NKT cells are important in coordinating functional interactions among decidual leukocytes at the maternal-fetal interface.

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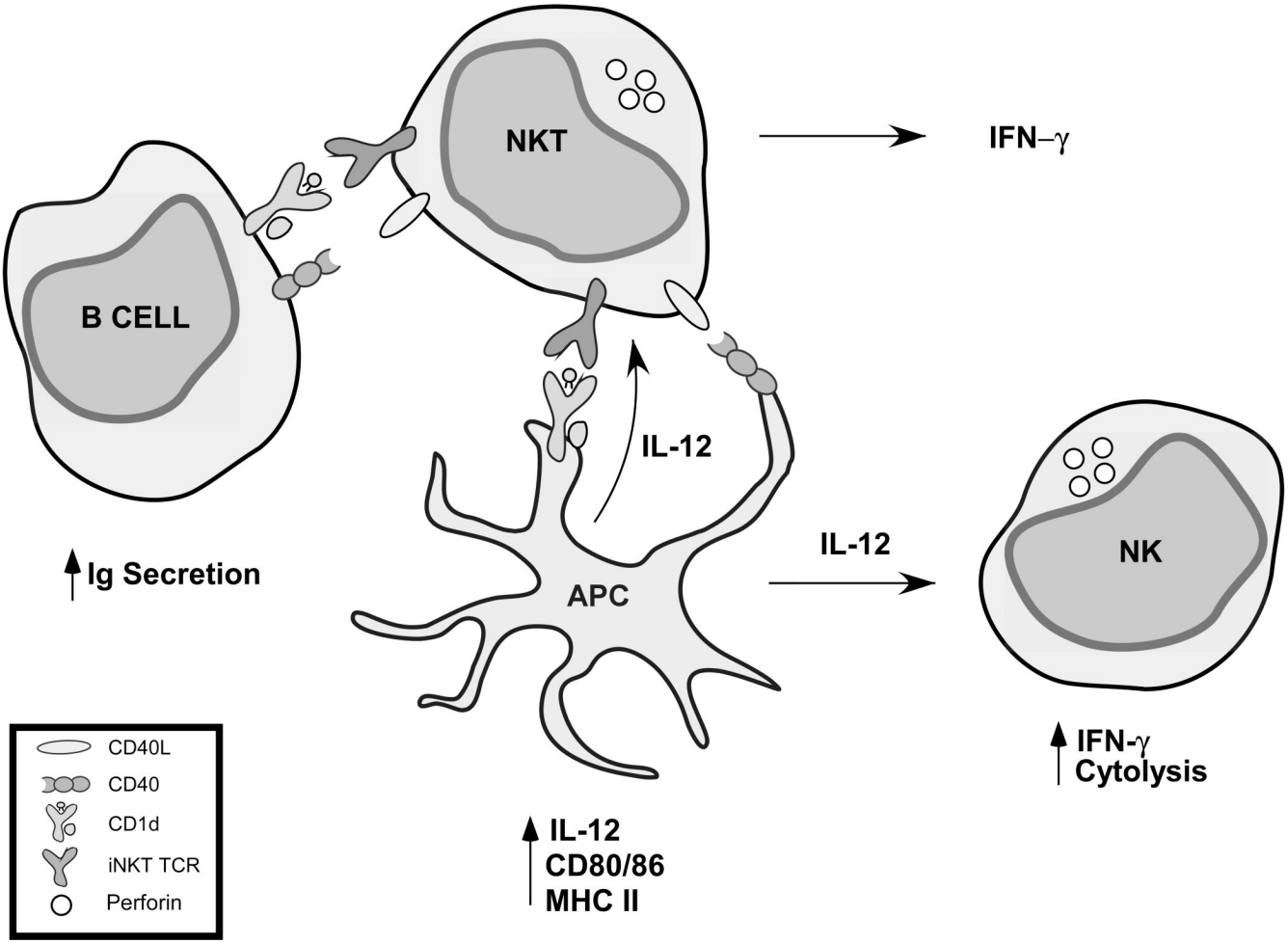

Figure 1.

Modulation of innate and adaptive immune responses by NKT cells. Activation of NKT cells leads to activation of a number of leukocyte subsets including B cells, dendritic cells (DCs), and NK cells.

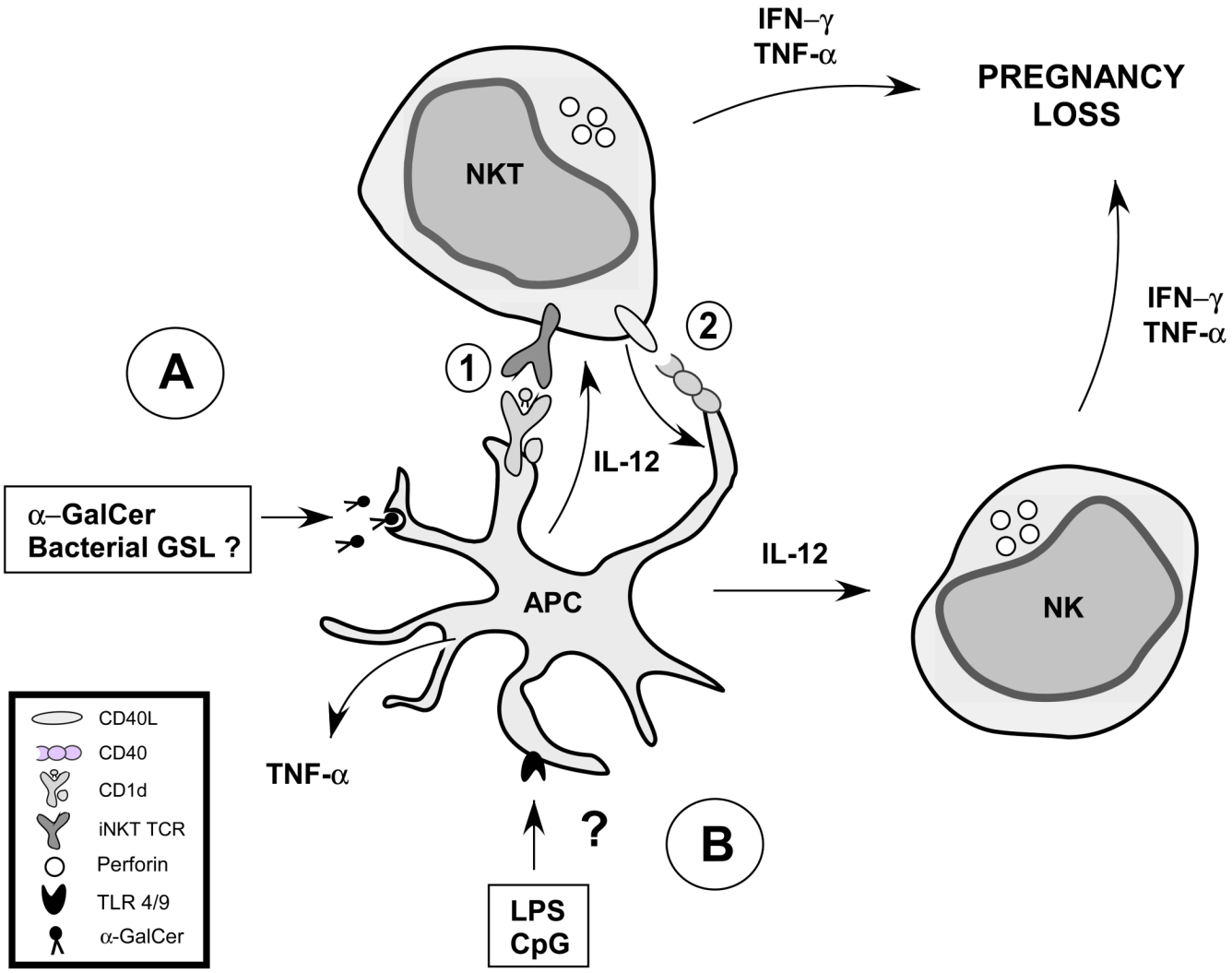

Figure 2.

Hypothetical model of NKT cells in immune-mediated pregnancy loss. NKT cells are activated directly by bacterial cell wall glycolipids, or they can be activated indirectly via LPS or CpG-stimulated APCs. Therefore, a hypothetical model may be constructed which integrates both of these pathways in immune-mediated pregnancy loss. A) Exogenous pathway. Pathogen-derived cell wall glycolipids are endocytosed by an APC and loaded onto CD1d. B) Endogenous pathway. Pathogen-derived TLR ligands such as LPS trigger TLR4 signaling on an APC (DC and/or macrophage) which induces loading of endogenous CD1d agonist ligands onto CD1d. Activation of NKT cells through TCR recognition of CD1d and agonist ligand leads to cytokine production and upregulation of CD40L (CD154). Cross-linking of CD40 expressed on the APC by CD40L in turn leads to APC activation and results in secretion of IL-12, which induces NK cell. IL-12 production leads to NK cell activation and subsequent production of IFN-γ and TNF.

Table I

Classification of NKT cells

	Type I (classical)		Type II (non-classical)	NKT-like		References
	Mouse	**Human**	**Mouse**	**Mouse**	**Human**	
TCR α	Vα14-Jα18 invariant	Vα24-Jα18 invariant	Diverse	Diverse	Diverse	(Cardell et al. 1995;Lantz and Bendelac 1994)
TCR β	Vβ8.2, Vβ7,Vβ2	Vβ11	Diverse	Diverse	Diverse	(Cardell et al. 1995;Lantz and Bendelac 1994; Porcelli et al. 1996)
CD1d-restricted	Yes	Yes	Yes	No	No	(Bendelac et al. 1995;Cardell et al. 1995;Exley et al. 1997)
Known Antigens[a]	αGalCer iGb3, GD3 αGlcUCer αGalUCer BbGL-II	αGalCer iGb3, GD3 αGlcUCer αGalUCer BbGL-II	Sulfatide	ND	ND	(Jahng et al. 2004;Kawano et al. 1997;Kinjo et al. 2006; Kinjo et al. 2005; Mattner etal. 2005; Spada et al 1998; Sriram et al 2005; Wu et al. 2003;Zajonc et al. 2005;Zhou et al. 2004)
Surface Markers	NK1.1 +/–[b] CD4, DN	CD161 +/– CD4, DN, CD8	NK1.1+/– CD4, DN	$NK1.1^+$ $DX5^+$	$CD56^+$ $CD161^+$	(Cardell et al. 1995;Hammond et al. 2001;Jahng et al. 2004;Spada et al. 1998)

[a] αGalCer, αGalactosylceramide; iGb3, isoglobotrihexosylceramide; GD3, disialoganglioside GD3; GSL; glycosphingolipid; αGlcUCer, α-glucuronosylceramide; αGalUCer, α-galacturonosylceramide; DN, double negative; ND, not determined

[b] the NK1.1 allele is expressed in only a few commonly used inbred strains such as C57BL/6, SJL, FVB/N, NZB, and NZW.