Role of human non-invariant NKT lymphocytes in the maintenance of type 2 T helper environment during pregnancy

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Abstract

The molecular and cellular mechanisms that generate the T_h2 cytokine environment necessary for the maintenance of pregnancy are still not fully understood. We herein show that the human decidua is highly enriched for TCR_{ab}^{+}CD161^{+} NKT cells. They express non-invariant antigen receptors encoded by diverse TCRV_{α}- and V_{β}-chain gene segments, thereby referred to as non-invariant NKT (non-iNKT) cells. In spite of their diverse TCR expression, they do not recognize fetal allo-antigens but specifically responded to CD1d-transfected cell lines. In contrast to the peripheral blood non-iNKT cells, the decidua-residing non-iNKT cells had a marked Th2 bias. In addition, they suppress the mixed leukocyte reaction directed against the paternal antigens. The T_{h}2 cytokines have been known to stimulate trophoblast outgrowth and invasion. Thereby, the non-iNKT cells residing in the decidual tissue may have a functionally important interaction with the villous and extravillous trophoblast cells expressing CD1d and may therefore play a pivotal role in successful pregnancy by inhibiting fetal rejection and enhancing placental growth. These findings may reflect one mechanism that is an essential component for the Th2 environment necessary for the maintenance of pregnancy.

Introduction

Allogeneic tissue grafts are typically rejected by immune responses directed against non-self-MHC. In contrast, the fetus, which resembles a semi-allogeneic graft, is accepted by the maternal immune system. Although the T_{h}2-type environment have been suggested to be a pivotal condition that permit the genetically distinct fetus to survive and develop within the mother, the precise cellular and molecular mechanisms underlying the maintenance of pregnancy and the induction of abortion remain unclear (1−4).

Several specialized mechanisms have evolved to help the fetus evade maternal immune attack. The fetal trophoblast cells that invade maternal uterine tissue at the site of implantation lack the surface expression of HLA-A and HLA-B antigens (5, 6), but selectively express HLA-C and non-classical HLA-E and HLA-G (7−9). They inhibit the cytolytic activity of NK cells through interaction with inhibitory receptors, namely Ig-like killer cell inhibitory receptor (KIR) and lectin-like KIRs (10−12). In addition, the expression of Fas ligand and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) by trophoblast cells may cause apoptosis of activated maternal T cells, which express Fas or TRAILR, respectively (13, 14). Moreover, the maternal CD4+CD25+ regulatory T cells are expanded and play an important role for the suppression of maternal immune response directed against the fetus (15). The indoleamine 2,3-deoxygenase, an enzyme that catabolizes tryptophan, indirectly suppresses maternal T cell activity by tryptophan deprivation (16).

Recently, the expression of CD1d molecules on villous and extravillous trophoblast cells has been reported (17). The CD1d molecule is specialized in the presentation of a limited set of glycolipids for recognition by NKT cells, a unique T cell subset expressing both TCRs and NK cell receptors (18). The placenta and the amnion are highly enriched for glyco-sphingolipids, many of which are sialic acid-containing...
gangliosides (19–22) that may be presented by CD1 and recognized by NKT cells (23–25). It is therefore important to elucidate the roles of CD1-restricted NKT cells that reside at the maternal–fetal interface during pregnancy. The most characterized NKT subset expresses an invariant TCRα chain (Vα14-Jα281 in mice and Vα24-JαQ in humans), recognizes a synthetic glycolipid, α-galactosylceramide (α-GalCer), and is referred to as invariant NKT (iNKT) cells (26). In addition to the iNKT cells, a population of CD1d-restricted NKT cells that express diverse TCRs, referred to as non-invariant NKT (non-iNKT) cells, have also been described (27, 28). In humans, non-iNKT cells have recently been characterized in the bone marrow and hepatatis C virus-infected liver (29, 30). The former is strongly T\(_h\)2 biased whereas the latter is T\(_h\)1 like, indicating that organ-specific mechanisms might dictate the functional capabilities of resident NKT cells. Although the iNKT cells present in the decidual tissue are involved in the maintenance of pregnancy in both mice and humans (17, 31), the spectrum of roles for non-iNKT cells in pregnancy remains to be defined.

This study demonstrates that human decidua was highly enriched for TCR\(\alpha\beta\)CD1d\(^+\) non-iNKT cells. In contrast to the peripheral blood non-iNKT cells, decidua-residing non-iNKT cells had a marked T\(_h\)2 bias, preferentially responded to CD1d, and suppress mixed leukocyte reaction (MLR). These findings indicate that decidua-residing non-iNKT cells may play an important role in successful pregnancy by inhibiting fetal rejection and enhancing placentl growth.

**Methods**

**Antibodies and reagents**

- Anti-CD161 (DX12), anti-CD56 (MY31), anti-CD3 (HI31a), and anti-CD28 (CD28.2) mAb and isotype control were obtained from BD PharMingen (San Diego, CA, USA).
- Anti-V\(\alpha\)24 (C15) and anti-V\(\beta\)11 (C21) mAbs were from Beckman Coulter (Marseille Cedex, France).
- Anti-TCR\(\alpha\)β (BMA031) mAb was from Caltag Laboratory (Burlingame, CA, USA).
- Anti-CD1d (55.3.1) mAb for blocking was kindly provided by Steven A. Porcelli (Albert Einstein College of Medicine). Anti-CD1d, b-, c- and d-transfected cell lines, C1R (B cell line) and BeWo (choriocarcinoma cell line) were transfected with pSR\(\alpha\)-neo expression vectors (pSR\(\alpha\)-neo-CD1a, b, c and d) were kindly provided by Steven A. Porcelli (Albert Einstein College of Medicine). To generate CD1a-, b-, c- and d-transfected cell lines, C1R (B cell line) and BeWo (choriocarcinoma cell line) were transfected with pSR\(\alpha\)-neo-CD1 by electroporation and selected with G418 (1 mg ml\(^{-1}\)).

**TCR\(\alpha\)\(\beta\)+CD1d\(^+\) NKT cell lines**

Second trimester decidua and peripheral blood from seven patients (14–21 weeks of gestation) undergoing elective abortion were collected. Informed consent was obtained for all cases. All the sampling and use of the tissues for this study were approved by the Saitama Medical University Ethics Committee. Decidual tissue was washed extensively in PBS supplemented with 50 \(\mu\)g ml\(^{-1}\) gentamicin and 250 \(\mu\)g ml\(^{-1}\) Fungizone amphotericin B (Invitrogen, San Diego, CA, USA) before mincing with sterile scissors. Decidual lymphocytes were released by digesting the tissue with 0.1% collagenase and 0.05% deoxyribonuclease I (Sigma, St Louis, MO, USA). PBMCs and decidual lymphocytes were isolated by Ficoll-Hypaque density gradient centrifugation (GE Healthcare). TCR\(\alpha\)\(\beta\)+CD1d\(^+\) NKT cells and V\(\alpha\)24/V\(\beta\)11\(^+\) NKT cells were sorted with a FACS Aria automated cell sorter (Becton Dickinson, San Diego, CA, USA). The sorted cells were stimulated with PHA (0.5 \(\mu\)g ml\(^{-1}\)) and irradiated (4500 cGy) autologous PBMCs. Cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated human serum, penicillin (100 U ml\(^{-1}\)), streptomycin (100 \(\mu\)g ml\(^{-1}\)) and 2 mM L-glutamine (Sigma). Cells were maintained at 37°C in 5% CO\(_2\) in a humidified atmosphere.

**EBV-transformed B cell lines**

EBV (B95-8) was kindly provided by M. Yasukawa (Ehime University, Ehime, Japan). CD56 \(^{-}\)CD161\(^-\) cells were isolated from PBMCs by negative magnetic cell sorting using FITC-conjugated anti-CD56 mAb, anti-CD161 mAb and FITC microbeads. EBV-transformed B cell lines were established from CD56 \(^{-}\)CD161\(^+\) cells by in vitro transformation as previously described (32).

**Monocyte-derived dendritic cells**

Induction of human monocyte-derived dendritic cells (DCs) was done as described (33). Further differentiation into mature DCs was induced by treatment with 1 \(\mu\)M prostaglandin E\(_2\) (Sigma) plus 20 ng ml\(^{-1}\) tumor necrosis factor \(\alpha\) (Primmune, Osaka, Japan).

**Transfectants**

cDNA encoding CD1 protein inserted with pSR\(\alpha\)-neo expression vectors (pSR\(\alpha\)-neo-CD1a, b, c and d) were kindly provided by Steven A. Porcelli (Albert Einstein College of Medicine). To generate CD1a-, b-, c- and d-transfected cell lines, C1R (B cell line) and BeWo (choriocarcinoma cell line) were transfected with pSR\(\alpha\)-neo-CD1 by electroporation and selected with G418 (1 mg ml\(^{-1}\)).

**MLR**

CD4\(^+\)CD45RO\(^-\) (naive) T\(_h\) cells were isolated from PBMCs by negative magnetic cell sorting using a CD4\(^+\) T cell isolation kit II (Miltenyi Biotec) and CD45RO microbeads. Allogeneic DCs (1.0 \(\times\) 10\(^5\)) were cultured with naive T\(_h\) cells (5.0 \(\times\) 10\(^4\)) in the presence of NKT cells (1.0 \(\times\) 10\(^4\)). After 5 days of culture, the cells were pulsed with \(^{3}\)H]-thymidine (1 \(\mu\)Ci per well) for 16 h and proliferative responses were measured as \(^{3}\)H]-thymidine incorporation.

**Reverse transcription–PCR**

Total RNA was extracted with the RNeasy Mini Kit (Qiagen, Valencia, CA, USA). Genomic DNA was digested and removed using an RNase-Free DNase kit (Qiagen). First-strand cDNA was synthesized using oligo (dT)\(_{12-18}\) primer (Invitrogen) and the Omniscript RT kit (Qiagen). Synthesized cDNA was subjected to PCR amplification for rearranged TCR\(\alpha\) genes with 29 5'-TCRAV family-specific oligonucleotides (V\(\alpha\)1-32) and a 3'-TCRAC (C\(\alpha\)) constant primer and for rearranged TCR\(\beta\) with 27 5'-TCRBV family-specific oligonucleotides (V\(\beta\)1-25) and a 3'-TCRBC (C\(\beta\)) constant primer as previously described (33).

**Real-time quantitative reverse transcription–PCR**

Transcripts were quantified by real-time quantitative PCR using an ABI PRISM 7900 sequence detector (Perkin-Elmer).
Applied Biosystems) with Applied Biosystems pre-designed TaqMan Gene Expression Assays and reagents according to the manufacturer’s instructions.

Measurement of cytokines
The cytokine levels in the culture supernatants were evaluated with ELISA (R&D Systems, Minneapolis, MN, USA).

Statistical analysis
Statistical analyses were performed using Student’s t-test. Values were considered statistically significant at a value of \( *P < 0.05 \).

Results
Human decidua is enriched for CD56+ and CD161+ NKT cells
To determine the frequency and phenotype of NKT cells in the decidua, the expression of NK cell markers by CD3+ T cells obtained from seven decidual tissues (14–21 weeks) was investigated in comparison to matched peripheral blood by flow cytometry. The frequencies of CD56+CD161+ and CD56−CD161+ T cells in decidual CD3+ T cells were much higher than the frequency seen in peripheral blood, while CD56−CD161− conventional T cells were much less frequent (Fig. 1A). Approximately 25–30 and 15% of CD3+ T cells were CD161+, in decidual tissue and peripheral blood, respectively (Fig. 1B). Most of the CD3+ T cells were TCRαβ+ both in the decidua and peripheral blood (Fig. 1A). The frequencies of TCRαβ+CD161+ T cells were higher than those seen in the peripheral blood. In addition to the high frequency of TCRαβ+CD161+ NKT cells in the decidua, the high frequency of Vα24/Vβ11+iNKT cells were also detectable in the decidua (0.4−0.7%, data not shown), the frequency were 5−10 times higher than those seen in the peripheral blood. Thereby, decidual T cells were enriched for NKT cells and most of which were TCRαβ+CD161+ NKT cells.

Decidual TCRαβ+CD161+ NKT cells expressing diverse TCRs do not recognize fetal allo-antigens
Previous results indicate that a major population of NKT cells in decidual tissue reveals a TCRαβ+CD161+ phenotype. To determine their role in regulating the immune response to the fetal allograft, TCRαβ+CD161+ NKT cells were purified using an automated cell sorter and a series of short-term T cell lines were expanded (Fig. 2A). Of the five decidual TCRαβ+CD161+ NKT cell lines tested, all consist of CD4+, CD8+ and double-negative sub-populations (data not shown). The origin of TCRαβ+CD161+ NKT cells in the decidua was of maternal origin, as determined by a PCR analysis for the Y-chromosome-specific sequence DYS14 (34).

![Fig. 1. Surface phenotype and frequency of CD56+ and/or CD161+ NKT cells in the decidua. (A) Representative dot plots \((n = 7)\) of CD3-gated lymphocytes stained with anti-CD56 and anti-CD161 mAbs. The numbers in quadrants indicate the percentage of cells in each. (B) Summary of CD56+ and/or CD161+ NKT cell frequencies as a percentage of CD3+ T cells in the decidua and in the periphery. The mean value of NKT frequencies is indicated by bar.](https://academic.oup.com/intimm/article-abstract/20/3/405/651938)
and by a PCR–restriction fragment length polymorphism analysis for the HLA-DR gene (data not shown). To determine whether there is a preferential use of TCRVα- or Vβ-chain element by decidual TCRβ+CD161+ NKT cells, we analyzed amplified cDNA using Vα- and Vβ-specific primers. Although some TCRVα- and Vβ-chain elements expressed in decidual TCRβ+CD161+ NKT cells differed from those of the peripheral blood in each individual, no consistent pattern emerged among the five individuals tested (Fig. 2B, data not shown). To assess the recognition nature of these TCRs in response to fetal antigens, TCRβ+CD161+ NKT cell lines were cultured with EBV-transformed B cell lines with paternal or maternal origin and assayed for proliferation (Fig. 2C). The proliferative responses of the conventional CD4+ T cells derived from maternal PBMCs to paternal EBV-transformed B cells were higher in magnitude than those from paternal PBMCs. The responses of the paternal CD4+ T cells to maternal EBV-transformed B cells were higher than those of maternal origin. In contrast, neither peripheral nor decidual TCRβ+CD161+ NKT cells responded to EBV-transformed B cell lines with either a paternal or maternal origin. These results suggest that decidual TCRβ+CD161+ NKT cells expressing diverse TCRs, namely the non-iNKT cell, do not recognize fetal allo-antigens.

**Decidual TCRβ+CD161+ NKT cells recognize CD1d molecules**

The decidual TCRβ+CD161+ NKT cell lines were next tested for responses to CD1a, b, c or d transfectants. To exclude the reactivity of Vα24 iNKT cells to CD1d, the TCRVα24-positive cells were depleted by negative magnetic bead sorting. The decidual TCRβ+CD161+ NKT cell lines responded specifically to CD1d-expressing C1R and BeWo transfectants and produced large amounts of IL-4 and IL-10 (Fig. 3A–D). There were no such responses to CD1a, b or c transfectants. In contrast, only a trace IFN-γ (< 150 pg ml⁻¹) was produced by decidual TCRβ+CD161+ NKT cells (data not shown). The recognition of CD1d required the sub-optimal doses of phorbol myristate acetate (PMA) (2 ng ml⁻¹) and IL-2 (0.1 U ml⁻¹) as described (35). The CD1d-specific responses were specifically blocked by anti-CD1d mAb (Fig. 3C and D). In contrast to the decidual TCRβ+CD161+ NKT cells, the CD1d-specific cytokine production was not detected in peripheral blood TCRβ+CD161+ NKT cells (data not shown). To assess the frequency of CD1d-restricted TCRβ+CD161+ NKT cells, the CD1d-specific cytokine production was depleted from TCRVβ+CD161+ NKT cells and produced large amounts of IL-4 and IL-10 but not IFN-γ (Fig. 4A–D, data not shown). Four of 10 cloned T cells that responded to CD1d expressed dual TCRVα and a single TCRVβ gene transcripts.
and others expressed a single TCRV\(\alpha\) and a single TCRV\(\beta\) gene transcripts, but a particular V\(\alpha\)- and V\(\beta\)-gene transcript was not observed. These results suggest that a high frequency of decidual TCR\(\alpha\)\(\beta\)CD1d\(^+\) NKT cells bearing different TCRs can recognize CD1d molecules.

Decidual TCR\(\alpha\)\(\beta\)CD1d\(^+\) NKT cells expressing diverse TCRs exhibit a T\(_{h}2\)-like phenotype

Next, the cytokine production by the series of decidual TCR\(\alpha\)\(\beta\)CD1d\(^+\) NKT cell lines (Fig. 5A) and clones (Fig. 5B) stimulated by plate-bound anti-CD3 plus soluble anti-CD28 mAb was assessed. For comparison purposes, a series of cognate TCR\(\alpha\)\(\beta\)CD1d\(^+\) NKT cells from the peripheral blood was also analyzed and the IL-4/IFN-\(\gamma\) ratios were compared. The decidual TCR\(\alpha\)\(\beta\)CD1d\(^+\) NKT cells revealed higher IL-4/IFN-\(\gamma\) ratios than those of peripheral blood TCR\(\alpha\)\(\beta\)CD1d\(^+\) NKT cells (Fig. 5A and B). The T\(_{h}2\)-biased cytokine profile was due to the reduced production of IFN-\(\gamma\). This tendency was also observed with PMA/ionomycin stimulation (data not shown). Next, the expressions of T-bet, GATA-3 and Foxp3 in TCR\(\alpha\)\(\beta\)CD1d\(^+\) NKT cell clones (\(n = 10\)) from two different individuals were examined by quantitative PCR (Fig. 5C). In comparison to the peripheral blood TCR\(\alpha\)\(\beta\)CD1d\(^+\) NKT cells, decidual TCR\(\alpha\)\(\beta\)CD1d\(^+\) NKT cells expressed higher levels of GATA-3, but lower T-bet. However, no significant difference was observed in the Foxp3 expression. These findings suggested that decidual TCR\(\alpha\)\(\beta\)CD1d\(^+\) NKT cells are more strongly T\(_{h}2\) polarized than those of the peripheral blood.

Decidual TCR\(\alpha\)\(\beta\)CD1d\(^+\) NKT cells suppress anti-fetal alloresponses

We next determined whether the decidual TCR\(\alpha\)\(\beta\)CD1d\(^+\) NKT cells have a capacity to suppress anti-fetal alloresponses. Mature DCs of paternal origin were cultured for 5 days with maternal naive Th\(_i\) in the absence or presence of decidual TCR\(\alpha\)\(\beta\)CD1d\(^+\) NKT cells or decidual V\(\alpha\)\(\delta\)24 iNKT cells. Decidual TCR\(\alpha\)\(\beta\)CD1d\(^+\) NKT cells suppressed anti-fetal alloresponses, whereas the decidual V\(\alpha\)\(\delta\)24 iNKT cells showed no inhibitory effect (Fig. 6A). To determine the mechanism for the suppression of the anti-fetal alloresponses, we investigated the proliferative response of naive Th\(_i\) in the presence of anti-IL-4, anti-IL-10 or anti-CD1d antibody. The addition of neutralizing anti-IL-4 antibody had little effect on the suppression of proliferation, but the neutralizing anti-IL-10 antibody or the blocking anti-CD1d antibody significantly reversed such suppression (Fig. 6B). These results indicate that the inhibitory effect of decidual TCR\(\alpha\)\(\beta\)CD1d\(^+\) NKT cells in the MLR was mediated by both CD1d recognition and subsequent IL-10 production.

Discussion

This study demonstrated that a population of CD1d-restricted NKT cells that is distinct from the V\(\alpha\)\(\delta\)24 iNKT cells accumulates in the decidual tissue, and they can contribute to the maintenance of a T\(_{h}2\) environment during pregnancy. The CD1d reactivity of decidual NKT cells did not correlate with a particular TCR\(\alpha\)- and V\(\beta\)-chain usage. The specificity for CD1d was demonstrated by the reduced response to CD1d transfectants in the CD1d blocking and by the failure to respond to CD1a, CD1b or CD1c transfectants. The CD1d...
expression has been reported in villous and extravillous trophoblasts from the early stages of pregnancy (17, 36). Therefore, the non-iNKT cells that reside in the decidua may have a functionally important interaction with the trophoblasts that express CD1d molecules.

The presence of a high frequency of non-iNKT cells in the decidua is intriguing because of their ability to rapidly produce large amounts of IL-4 on engagement of the TCRs. This characteristic correlates with the observations that predominantly Th2 cytokines help the maintenance of pregnancy, and a shift toward a Th1 cytokine milieu is associated with reproductive failure (37–39). In addition, the accumulation of non-iNKT cells in the decidua indicates that these cells are actively recruited to and/or the population is expanded at this site. Although the decidual non-iNKT cells express diverse TCRs, they do not recognize fetal alloantigens. This finding indicates that their accumulation might occur due to the CD1d expression on trophoblasts rather than the recognition of the fetal antigens. Indeed, it is possible that the pregnancy-associated hormones such as estrogen and progesterone may have various effects on these cells (40).

The lipid antigens recognized by NKT cells in the maternal–fetal interface have not yet been determined. It has been suggested that monosialoganglioside GM3 (GM3) is a major ganglioside in the placenta and is predominant during the middle stage of pregnancy, while disialoganglioside GD3 (GD3) is expressed in the late stage of gestation (20). In mouse placenta, the increased expression of an invariant TCRα chain encoded by Vα11 and Jα281 gene segments is tightly correlated with the middle stage of gestation when GM3 predominates (24). In contrast, the TCRβ11 sequence change after which GD3 expression predominates during the late stage of pregnancy. These findings of earlier studies indicate that TCR sequences seem to be associated with changes in the ganglioside composition according to the stage of pregnancy. The initial hypothesis was that the decidual NKT cells may selectively express the limited TCRαβ chains, recognize the gangliosides and play a certain role in the pregnancy. Contrary to expectations, the decidual non-iNKT cells do not recognize GM3, GD3 and α-GalCer presented by CD1d. In addition, they do not express a biased TCRαβ and Vβ gene transcript. Thereby, it is possible that the decidua-residing NKT cells, which express diverse TCRs, may recognize other lipid antigens specifically modified during pregnancy and may play a decisive role in the regulation of maternal–fetal immune responses. It is also possible that the decidual non-iNKT cells expressing different TCRs may recognize sequentially different lipids according to the stage of pregnancy. The endogenous ligands recognized by these T cells are currently under investigation.

The iNKT cells have been reported to develop in the fetus at an early stage of embryogenesis (41). In addition, the
fetal microchimerism can be detected in the maternal circulation (34). Therefore, it is of great interest to clarify whether the decidua-residing non-iNKT cells are of maternal or fetal origin. The current finding indicates that the non-iNKT cells residing in the decidual tissue are entirely maternally derived. However, the details of the development of this population remain unresolved.

In a recent study, the decidual iNKT cells exhibited a marked T_h2-biased phenotype and a striking polarization toward granulocyte macrophage colony-stimulating factor production (17). However, the decidual non-iNKT cells herein presented exhibited a clear bias toward IL-4 and IL-10 secretion compared with the peripheral blood non-iNKT cells. As the T_h2 cytokines have been known to stimulate trophoblast outgrowth and invasion (42–44), the decidual non-iNKT cells may play an important role in the implantation and trophoblast invasion by producing T_h2 cytokines. The difference in the cytokine production between the decidua-residing iNKT cells and non-iNKT cells suggests that these distinct populations play different roles in the maintenance of pregnancy.

The CD1 molecules present glycolipids to T cells. Of the five CD1 isoforms identified so far (CD1a, b, c, d and e), all are present in humans, while mice and rats only possess CD1d (45). This observation indicates that there may be important functional differences between the lipid antigen-presenting milieu in humans and mice. Thereby, exploring the function of decidual non-iNKT cells in several animals would provide important insight into the role of non-iNKT cells for the maintenance of pregnancy.

There are many general adaptations of maternal immune system during pregnancy. The CD4^+CD25^+ regulatory T cells are required for maternal immune systems to tolerate fetal allografts. The MLR-suppressing activity observed in decidual non-iNKT cells in several animals would provide important insight into the role of non-iNKT cells for the maintenance of pregnancy.

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Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>DC</td>
<td>dendritic cell</td>
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<tr>
<td>GD3</td>
<td>disialoganglioside GD3</td>
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<td>GM3</td>
<td>monosialoganglioside GM3</td>
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<tr>
<td>α-GalCer</td>
<td>α-galactosylceramide</td>
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<tr>
<td>iNKT</td>
<td>invariant NKT</td>
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<tr>
<td>KIR</td>
<td>killer cell inhibitory receptor</td>
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<td>MLR</td>
<td>mixed leukocyte reaction</td>
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References
