

## Natural killer T cells : a bridge to tolerance or a pathway to rejection?

Jukes, John-Paul; Wood, Kathryn J; Jones, Nick D

DOI:

[10.1097/01.tp.0000280551.78156.ac](https://doi.org/10.1097/01.tp.0000280551.78156.ac)

License:

Other (please specify with Rights Statement)

*Document Version*

Early version, also known as pre-print

*Citation for published version (Harvard):*

Jukes, J-P, Wood, KJ & Jones, ND 2007, 'Natural killer T cells : a bridge to tolerance or a pathway to rejection?', *Transplantation*, vol. 84, no. 6, pp. 679-81. <https://doi.org/10.1097/01.tp.0000280551.78156.ac>

[Link to publication on Research at Birmingham portal](#)

### **Publisher Rights Statement:**

This is a non-final version of an article published in final form in *Transplantation*, Volume 84(6), 27 September 2007, pp 679-681.

Eligibility for repository : checked 13/06/2014

### **General rights**

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

### **Take down policy**

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact [UBIRA@lists.bham.ac.uk](mailto:UBIRA@lists.bham.ac.uk) providing details and we will remove access to the work immediately and investigate.

# **Natural Killer T Cells: A Bridge To Tolerance Or A Pathway To Rejection?**

**John-Paul Jukes<sup>1,3</sup>**

**Kathryn J Wood**

**Nick D Jones<sup>2</sup>**

**Transplantation Research Immunology Group,  
Nuffield Department of Surgery,  
University of Oxford,  
John Radcliffe Hospital,  
Oxford OX 3 9DU  
UK**

<sup>1</sup> J-PJ holds a graduate studentship funded by the Medical Research Council, UK

<sup>2</sup> NDJ is a Kidney Research UK Senior Fellow

<sup>3</sup> To whom correspondence should be addressed: [john-paul.jukes@nds.ox.ac.uk](mailto:john-paul.jukes@nds.ox.ac.uk)

## **Word Counts**

<b>Abstract:</b>	<b>52</b>
<b>Text:</b>	<b>1163</b>
<b>Table/Figure:</b>	<b>96</b>

**Keywords: NKT Cells, Transplantation, Tolerance, Rejection**

## **Abstract**

Over the past twenty years Natural Killer T (NKT) cells have been shown to play an important role in both innate and adaptive immune responses. In this review the potential role of NKT cells in transplantation will be discussed, in particular, their role in rejection and the induction of a state of tolerance.

Natural Killer T (NKT) cells represent a small but significant population of T lymphocytes (<1%) that are reactive to glycolipids presented in the context of the major histocompatibility complex (MHC) class I-like molecule, CD1d (Table 1). Although there appears to be a number of subtypes of NKT cells the most extensively characterised are the invariant or iNKT cells which are defined by the expression of a semi-invariant T cell receptor (TCR) composed of a V $\alpha$ 14-J $\alpha$ 18 chain associated with V $\beta$ 8.2, V $\beta$ 7, V $\beta$ 2 chains in mice and V $\alpha$ 24-J $\alpha$ 18 associated with V $\beta$ 11 in humans. Since the discovery of NKT cells in the mid 1980's it has become apparent that these cells have the potential to coordinate sophisticated immune responses, 'bridging' the interface of innate and adaptive immunity (1-3). The focus of this brief review is to discuss the potential role of NKT cells in transplantation immunology, and in particular, their role in rejection and the induction of a state of tolerance.

Upon activation NKT cells can mediate rapid and sustained production of a plethora of cytokines capable of impacting both innate and acquired immune responses (Fig.1A). For example,  $\alpha$ -galactosylceramide ( $\alpha$ GalCer), to date the most potent iNKT cell ligand, induces cytokine production within hours (4). It is believed that the distribution of NKT cells and the microenvironment in which they exist play a pivotal role in the generation of different effector populations. Indeed in mice NKT cells account for 30% of lymphocytes in the liver and <1% in spleen, blood and lymph nodes.

To date, two models of NKT cell activation have been proposed (5). The first suggests that NKT cells may exist in two subtypes, Th1-like NKT cells and Th2-like NKT cells which preferentially drive Th1 immunity and Th2/tolerogenic responses, respectively. The second model suggests that NKT cells are a homogeneous population that attain functional phenotypes according to the different cytokine microenvironment in which they are activated and the affinity of TCR for the activating ligand (Fig.1A). In addition to the conundrum of generating both pro- and anti-inflammatory cytokines, the downstream leukocyte populations that are activated as a result of NKT cell stimulation adds another level of complexity to the puzzle.

Whilst there is evidence to suggest a role for NKT cells in the immune response to a transplant, it remains unclear whether or not they can recognise alloantigens directly as it has been shown that CD1d,

unlike classical MHC molecules, is not polymorphic (6). NKT cells may be activated during transplantation by the milieu of cytokines generated by non-specific inflammation. Furthermore, the presentation of iGb3, or other as yet unknown glycolipids, following tissue damage may promote activation of NKT cells in a CD1d-dependent manner. NKT cells may promote rejection or tolerance depending on their response to these stimuli,

In two models of streptozotocin induced diabetes, NKT cells have been shown to promote graft loss of intraportally transplanted Islets of Langerhans. Firstly, allogeneic islets transplanted into NKT<sup>-/-</sup> mice showed a two fold increased survival time compared to those transplanted to WT mice (7). Moreover, in diabetic WT mice normoglycaemia can only be achieved with the transplantation of 400 syngeneic islets, compared to only 100 in NKT<sup>-/-</sup> mice (8). The process of graft damage was shown to be mediated by interferon- $\gamma$  (IFN $\gamma$ ) release from NKT cells that subsequently led to the recruitment of neutrophils, resulting in islet loss. However, despite these examples, most available evidence suggests that in the context of transplantation NKT may dampen rather than exacerbate rejection.

Oh *et al*, reported that in CD1d<sup>-/-</sup> mice transplanted with sex mismatched skin grafts, rejection occurred in an accelerated fashion compared to mice with an intact population of NKT cells. Furthermore, activation of NKT cells with  $\alpha$ Galcer significantly increased graft survival in wild-type mice (9). In addition, there are also a number of reports that suggest that NKT cells may facilitate the induction of tolerance to allografts. Seino *et al* (2001) demonstrated that cardiac allografts were accepted in mice treated with anti LFA-1/ICAM-1 or CD28/B7 monoclonal antibodies (mAbs) but were rejected in iNKT<sup>-/-</sup> mice that received the same mAbs (10). NKT cells have also been reported to be essential for the induction of tolerance in a number of other models, namely, cardiac (11) rat xenograft islets (12) and corneal transplantation (13). However, it should be noted that NKT cell involvement in tolerance induction may be reagent and/or transplant specific as in other models, tolerance induction was unperturbed by the absence of these cells (14).

In all the experimental models outlined above, the beneficial role of NKT cells in prolonging graft survival has been attributed to their production of various cytokines. Skin and cardiac allograft survival was shown to involve increased interleukin-10 (IL-10) production, which was decreased in CD1d<sup>-/-</sup> mice

(9,11,15). The production of IFN $\gamma$  by NKT cells remains uncertain, for example, Toyofuku et al found high levels of IFN $\gamma$  and decreased expression of IL-4 during the rejection process in NKT cell intact mice compared to NKT $^{-/-}$  mice (7). However, IFN $\gamma$  was found to be required for tolerance induction to cardiac allografts. NKT $^{-/-}$  mice reconstituted with IFN $\gamma^{-/-}$  cells rapidly rejected allografts which survived with the adoptive transfer of NKT cells (10). These data suggest that although there are many gaps in our current understanding of how tolerance may be influenced by NKT cells, there is evidence to suggest that NKT cells may primarily serve to aid the generation of CD4 $^{+}$ CD25 $^{+}$  T cells with regulatory activity (Treg). Indeed, this has been demonstrated in a model of type-1 diabetes (T1D) where the activation of iNKT with  $\alpha$ GalCer conferred protection from T1D which was dependent on IL-4 production and the presence of Treg (17).

Although there is controversy regarding the roles of NKT cells in tolerance and rejection these findings may be explained by subtle changes in the Th1/Th2 bias of NKT cells. Weak antigenicity and therefore weak adaptive responses, may allow NKT greater control of downstream events, preferentially producing tolerogenic cytokines (9). This may also be the case, as shown in other models where blockade of co-stimulatory, accessory or adhesion molecules is used as a strategy for inducing operational tolerance to allografts. However in cases where a Th1 bias exists and there is a strong allogeneic response, the contribution of NKT cells may be overshadowed as allogeneic T cells dominant the alloantigen driven response (Fig.1B).

The involvement of NKT cells in the immune response after transplantation highlights the function of these dynamic lymphocytes. Whilst it remains unclear as to the precise role of NKT cells in early inflammation, rejection and tolerance, elucidating the precise mechanisms by which they contribute and influence these processes is an important question in modern transplant immunology. There is no doubt that the potential impact NKT cells have on the activation of multiple arms of the immune responses is impressive. It will be important to determine the phenotype and functional properties of each NKT cell population that are responsible for promoting tolerance or rejection and to dissect these signalling pathways in more detail. With the recent discovery of glycolipid analogues that are capable of

preferentially driving NKT cells towards a Th1/Th2 bias, it is an exciting time for understanding how we can drive NKT cells to an altruistic pathway.

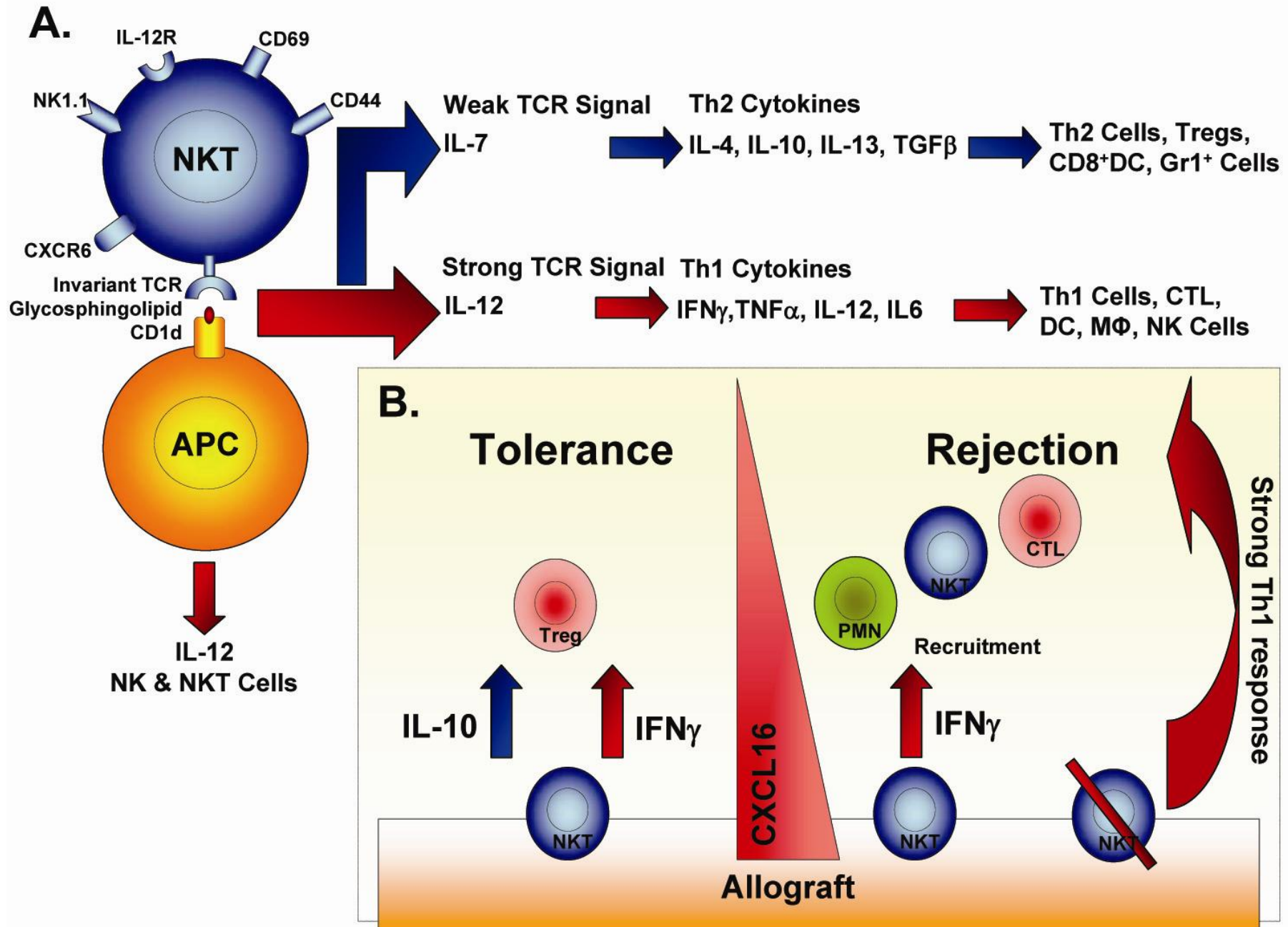
**Table i.**

TCR	Glycosphingolipid Ligands	Cytokine Production	Ref.
<b>NKT Cell TCR</b> Vα14 Jα18 associated with Vβ8.2, 7, 2	α-galactosylceramide (αGalCer)	Th1/Th2 responses (IFNγ, IL-4)	(18)
	└ analogues of αGalCer      OCH	preferential Th2 response (IL-4)	(19)
	C-glycoside	preferential Th1 response (IFNγ)	(20)
	<u>Bacterial Recognition</u>	Th1/Th2 responses (IFNγ, IL-4) but reduced compared to αGalCer	(21)
	<i>Sphingomonas</i> derived:		
	α-glucuronosylceramide (GSL-1 )		
	α-galacturonosylceramide (GS-1')		
	<u>Endogenous Recognition</u>		
	Isoglobotrihexosylceramide (iGb3)		(22)



**Table i. NKT cells recognise a number of ligands presented by CD1d.** These ligands can range from synthetic compounds to bacterial and endogenous glycosphingolipids. Following TCR engagement a variety of Th1 and Th2 cytokines can be released.







**Figure 1 A.** Activation of NKT cells results in the produce of a number of cytokines which can affect both innate and adaptive immune responses.

**Figure 1 B.** Following transplantation NKT cells may become activated by increased iGb3 presentation and the local cytokines microenvironment. Other factors that may influence NKT cell activation include the strength of TCR signalling, unknown glycolipid ligands and the site of transplantation.

## Reference List

1. Budd RC, Miescher GC, Howe RC, Lees RK, Bron C, MacDonald HR. Developmentally regulated expression of T cell receptor beta chain variable domains in immature thymocytes. *J Exp Med* 1987;166: 577-582.
2. Ceredig R, Lynch F, Newman P. Phenotypic properties, interleukin 2 production, and developmental origin of a "mature" subpopulation of Lyt-2-. *Proc Natl Acad Sci U S A* 1987;84: 8578-8582.
3. Fowlkes BJ, Kruisbeek AM, Ton-That H et al. A novel population of T-cell receptor alpha beta-bearing thymocytes which predominantly expresses a single V beta gene family. *Nature* 1987;329: 251-254.
4. Kawano T, Cui J, Koezuka Y et al. CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosylceramides. *Science* 1997;278: 1626-1629.
5. Godfrey DI, Kronenberg M. Going both ways: immune regulation via CD1d-dependent NKT cells. *J Clin Invest* 2004;114: 1379-1388.
6. Balk SP, Bleicher PA, Terhorst C. Isolation and characterization of a cDNA and gene coding for a fourth CD1 molecule. *Proc Natl Acad Sci U S A* 1989;86: 252-256.
7. Toyofuku A, Yasunami Y, Nabeyama K et al. Natural killer T-cells participate in rejection of islet allografts in the liver of mice. *Diabetes* 2006;55: 34-39.
8. Yasunami Y, Kojo S, Kitamura H et al. Valpha14 NK T cell-triggered IFN-gamma production by Gr-1+CD11b+ cells mediates early graft loss of syngeneic transplanted islets. *J Exp Med* 2005;202: 913-918.

9. Oh K, Kim S, Park SH et al. Direct regulatory role of NKT cells in allogeneic graft survival is dependent on the quantitative strength of antigenicity. *J Immunol* 2005;174: 2030-2036.
10. Seino KI, Fukao K, Muramoto K et al. Requirement for natural killer T (NKT) cells in the induction of allograft tolerance. *Proc Natl Acad Sci U S A* 2001;98: 2577-2581.
11. Jiang X, Shimaoka T, Kojo S et al. Cutting edge: critical role of CXCL16/CXCR6 in NKT cell trafficking in allograft tolerance. *J Immunol* 2005;175: 2051-2055.
12. Ikehara Y, Yasunami Y, Kodama S et al. CD4(+) V $\alpha$ 14 natural killer T cells are essential for acceptance of rat islet xenografts in mice. *J Clin Invest* 2000;105: 1761-1767.
13. Sonoda KH, Taniguchi M, Stein-Streilein J. Long-term survival of corneal allografts is dependent on intact CD1d-reactive NKT cells. *J Immunol* 2002;168: 2028-2034.
14. Beilke JN, Kuhl NR, Van KL, Gill RG. NK cells promote islet allograft tolerance via a perforin-dependent mechanism. *Nat Med* 2005;11: 1059-1065.
15. Jiang X, Kojo S, Harada M, Ohkohchi N, Taniguchi M, Seino KI. Mechanism of NKT Cell-Mediated Transplant Tolerance. *Am J Transplant* 2007;7: 1482-1490.
16. Sawitzki B, Kingsley CI, Oliveira V, Karim M, Herber M, Wood KJ. IFN-gamma production by alloantigen-reactive regulatory T cells is important for their regulatory function in vivo. *J Exp Med* 2005;201: 1925-1935.
17. Ly D, Mi QS, Hussain S, Delovitch TL. Protection from type 1 diabetes by invariant NK T cells requires the activity of CD4+CD25+ regulatory T cells. *J Immunol* 2006;177: 3695-3704.

18. Crowe NY, Uldrich AP, Kyparissoudis K et al. Glycolipid antigen drives rapid expansion and sustained cytokine production by NK T cells. *J Immunol* 2003;171: 4020-4027.
19. Miyamoto K, Miyake S, Yamamura T. A synthetic glycolipid prevents autoimmune encephalomyelitis by inducing TH2 bias of natural killer T cells. *Nature* 2001;413: 531-534.
20. Schmieg J, Yang G, Franck RW, Tsuji M. Superior protection against malaria and melanoma metastases by a C-glycoside analogue of the natural killer T cell ligand alpha-Galactosylceramide. *J Exp Med* 2003;198: 1631-1641.
21. Kinjo Y, Wu D, Kim G et al. Recognition of bacterial glycosphingolipids by natural killer T cells. *Nature* 2005;434: 520-525.
22. Zhou D, Mattner J, Cantu C, III et al. Lysosomal glycosphingolipid recognition by NKT cells. *Science* 2004;306: 1786-1789.