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# The importance of B cell CD1d expression for humoral immunity

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## Abstract

It was reported over a decade previously that CD1d-restricted Natural Killer T (NKT) cells could interact with CD1d-expressing B cells and facilitate antibody secretion. Since then, several studies have observed that NKT cells can provide B-cell help for production of antibody against model and pathogen-derived glycolipids, carbohydrates and proteins. In regard to T cell-dependent protein antigens, it is still not entirely clear to what extent cognate interactions between CD1d-expressing B cells and NKT cells contribute to initial and long-lived B-cell responses that are characteristic of such antigens. In this editorial, we review evidence that demonstrates that NKT cells provide CD1d-dependent cognate and non-cognate forms of B-cell help following immunization with protein antigen. Elucidating these mechanisms will be important for harnessing NKT cells during vaccination.

### Keywords

B lymphocyte; CD1d; NKT cell; cognate interactions; antibody

Understanding the full range of T cell help available to B cells is required if vaccine technology is to progress such that humoral immunity can be appropriately tailored to produce the desired blend of memory B cells and long-lived plasma cells that encode pathogen-neutralizing antibody (Ab).

Several groups have examined the effects of NKT cells on humoral immunity since a 2003 report that human NKT cells could enhance CD1d-dependent secretion of Ab by autologous B cells [1]. It is now appreciated that CD1d-restricted Type I Natural Killer T (NKT) cells provide Ag-specific B cell help leading to sustained production of high affinity IgG via B cell memory and the generation of long-lived plasma cells (reviewed in [2,3]). This has been observed for several model Ags, and those derived from viruses including Influenza and Herpes simplex virus 1 [2–4]. NKT cells enhance Ab responses to Ags from several bacteria including *Borrelia hermsii*, *Clostridium tetani*, *Bacillus anthracis* and *Streptococcus pneumoniae* (also reviewed in [2,3]).

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The mechanisms by which B cells and NKT cells interact and the implications for the ensuing humoral immune response are incompletely understood. B cells may encounter glycolipid, carbohydrate, or protein Ag in conjunction with NKT-activating CD1d ligand and thus receive 'help' for humoral immunity. Evidence indicates that the mechanisms regulating the immune response to different types of Ag depend on direct (cognate) molecular interactions and indirect (non-cognate) interactions between B cells and NKT cells. For T-independent Ag, B/NKT interactions appear to be cognate. For T-dependent Ag, B/NKT interactions are often described as non-cognate, which as will be discussed, is likely an over-simplification. Herein, it is proposed that during T-dependent humoral immune responses an initial CD1d-dependent cognate interaction between B cells and NKT cells contributes to NKT activation. NKT-derived B cell help is then provided in a cognate and non-cognate manner.

## Glycolipid Ag

In one model, the BCR has affinity for a CD1d-binding glycolipid (or a hapten associated with it) and internalizes the molecule to Ag-processing endosomes whereby CD1d is loaded with the ligand and then presented on the cell surface. Therefore the invariant TCR expressed by Type I NKT cells engages the CD1d/glycolipid complex, is activated, and subsequently provides help to the B cell. A CD1d-binding glycolipid known as α-galactoslyceramide (α-GC), or a derivative thereof, has been used in several studies. This glycolipid consists of an acyl chain and a sphingosine chain coupled to a galactose head-group. The α-GC molecule is loaded into hydrophobic pockets in CD1d, orienting the sugar head-group for recognition by the NKT TCR [5].

Lang and colleagues observed that the BCR on CD1d-transfected A20IIA1.6 cells could capture complexes containing biotin-α-GC leading to a 1000-fold enhanced CD1d-dependent activation of an NKT hybridoma [6]. In two subsequent studies, Leadbetter and colleagues demonstrated that immunization of mice with NP-hapten-linked α-GC led to CD1d-dependent and NP-specific Ab responses [7,8]. In those studies, there was a notable absence of Ab class switch and B cell memory. However, it was apparent that responses were dependent on CD1d and B7.1 expression by B cells. Furthermore, in the latter of those studies, a sub-set of activated NKT cells differentiated into $CD44^{hi}/CXCR5^{+}/PD\text{-}1^{+}$ and IL-21-secreting NKT cells, reminiscent of T follicular helper ($T_{fh}$) cells and were thus designated $NKT_{fh}$ cells [8]. Two other studies published at that time also reported differentiation of $NKT_{fh}$ cells [9,10]. The interaction between B cells and NKT cells was therefore described as 'cognate' and is consistent with available data.

## Carbohydrate Ag

A large carbohydrate Ag will normally stimulate a classical T-independent Ab response in which IgM is the dominant Ab class produced and there is little or no isotype switching and/or differentiation of memory B cells. Another model is therefore one in which carbohydrate Ag and CD1d-binding α-GC are co-administered. The BCR is specific for the carbohydrate Ag, but uptake of α-GC leads to CD1d-dependent NKT activation. In this model, there will be CD1d/NKT-dependent Ab class switch and differentiation of memory B

cells whereby the Ab response has characteristics of T-dependent humoral immunity. The Lang group previously observed that co-immunization of B6 mice with NP-Ficoll and α-GC led to modest class switch, whereby IgG1 was produced in addition to IgM and IgG3 [11]. IgG1 production was not observed in $CD1d^{-/-}$ mice or in the absence of α-GC. More recently, Bendelac and colleagues immunized mice with liposomes presenting *Streptococcus pneumoniae*-derived capsular polysaccharide and α-GC and observed isotype switch, Ab affinity maturation and B cell memory [12]. Conditional ablation of $CD1d^{+/+}$ B cells also confirmed the importance of B cell CD1d in the process. It therefore appears that a cognate CD1d-dependent B/NKT interaction occurs following co-immunization with carbohydrate Ag and α-GC. Further study is needed to elucidate the mechanisms by which NKT cells provide help to B cells in this context, but $NKT_{fh}$ cells may be part of the process [12].

## Protein Ag

The most intensively studied mechanism is where a T-dependent protein Ag and α-GC are co-administered and Ab-specific for the protein Ag is enhanced. In this model professional APCs including dendritic cells (DCs) capture both Ag and the adjuvant (α-GC) and present peptide and glycolipid in the context of MHC class II and CD1d respectively. Classical CD4 T cells are primed and NKT cells may be activated and/or differentiate into $NKT_{fh}$ cells. B cells also capture, process and present both protein Ag and glycolipid thus receiving classical ($T_h$) and non-classical (NKT) help. Capture of both protein Ag and glycolipid by B cells will also be facilitated by physical linkage of the two or incorporation into liposomes.

Available evidence suggests that DC CD1d make an initial contribution to NKT-enhanced humoral immunity. Mixed bone marrow chimeric mice in which 50% of DCs expressed diphtheria toxin receptor (DTR) under control of the CD11c promoter were used to temporarily ablate $CD1d^{+/+}$ DCs leaving $CD1d^{+/+}$ or $CD1d^{-/-}$ DCs [13]. In that study DC CD1d did not play a significant role in NKT-enhanced Ab responses unless all DCs were $DTR^{+/+}$ and ablated. Under those circumstances, NKT-enhanced Ab responses were delayed [13]. However a Cre-Lox system designed to ablate only $CD1d^{+}$ DCs, showed definitively that DC CD1d contributes to NKT-enhanced Ab responses [12].

Evidence that B cell CD1d expression regulates NKT-enhanced humoral immunity is quite clear. Lang and colleagues demonstrated by adoptive transfer of $CD1d^{+/+}$ and $CD1d^{-/-}$ B cells into μMT recipient mice that CD1d expression was necessary for NKT-enhanced responses against protein Ag [14]. The requirement for CD1d expression by B cells was further demonstrated using liposomes containing protein Ag and α-GC as immunogens [15]. Tonti and colleagues have observed that NKT-enhanced humoral immunity can require cognate and non-cognate B/NKT interactions [10,16]. The reasons for the differing results are not entirely clear, but experimental system, Ag, dose (of Ag and α-GC), and route of immunization, could influence the relative dependency on B cell CD1d expression.

Working with human B cells, the van den Elzen group showed that retinoic acid and α-GC led to down-regulation of CD1d expression by B cells, suggesting a confined time-window for physical B/NKT activation [17]. In mice, Batista, Vinuesa and colleagues used intra-vital microscopy to show that fluorescently-labeled hen egg lysozyme (HEL)-specific MD4 B

cells and NKT cells interacted directly for 4 – 50 minutes after immunization, again supporting a direct but time-constrained interaction [9]. Available evidence therefore leans heavily towards an initial direct, cognate interaction between $CD1d^{+/+}$ B cells and NKT cells.

At this time it remains unclear exactly how the NKT cell communicates back to the B cell to provide helper signals. Using a mixed bone marrow chimera approach, Shah and colleagues demonstrated that $CD40L^{-/-}$ NKT cells were equally capable to their wild type counterparts in stimulating an NKT-enhanced Ab response [18]. ICOS could not be studied in this manner because it is required for NKT cell development, but in an in vitro study, it was shown that ICOS was required for activation of marginal zone B cells [19]. Since marginal zone B cells are associated with T-independent Ab responses, it remains unclear what the role of NKT cell ICOS is during T-dependent responses. Terhorst and colleagues demonstrated that signaling lymphocyte activation molecule (SLAM)–associated protein (SAP) expressed by NKT cells was dispensable for production of IgM reactive to protein Ag, but contributory to differentiation of germinal center B cells, Ig class switch and affinity maturation [20].

Evidence supports the notion that NKT-derived soluble factors play a role in T-dependent B cell responses. NKT-derived IL-4 and IFNγ do appear to play some limited role in shaping Ig subclass production against anthrax toxin [21]. Shah and colleagues recently reported that a sub-set of NKT cells could secrete plasma cell survival factors BAFF and APRIL which collectively were required for plasma cell survival, but dispensable for B cell memory [22]. It is also likely that $NKT_{fh}$-derived IL-21 contributes to T-dependent IgG1 production [9].

The evidence therefore demonstrates that cognate interaction of B cells and NKT cells is at the least, a contributor to NKT-stimulated enhancement of T-dependent humoral immunity. The time window for this interaction may be limited, and is likely supplemented by non-cognate signals which contribute to B cell memory and plasma cell longevity.

## Concluding Remarks

In conclusion, direct interaction between B cell CD1d molecules and the TCR of Type I NKT cells is at least contributory in stimulating long-lived, T-dependent humoral immunity. The significance of B cell CD1d may go beyond the realm of vaccine studies, since its importance has been demonstrated in maintaining NKT homeostasis in lupus patients [23] and in production of Abs against blood group Ags [24]. Therefore, the initial cognate interaction should feature in models whereby B cells receive classical as well as non-classical B cell help.

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## Abbreviations used

| | |
|---|---|
| **α-GC** | α-galactosylceramide |

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| **APRIL** | A Proliferation-Inducing Ligand |
| **BAFF** | B cell-Activating Factor |
| **BCR** | B Cell antigen Receptor |
| **ICOS** | Inducible T cell COStimulator |
| **NKT** | Natural Killer T cell |
| **NKTfh** | Follicular Helper Natural Killer T cell |
| **NP** | Nitrophenol hapten |
| **SAP** | SLAM-Associated Protein |
| **SLAM** | Signaling Lymphocyte Activation Molecule |
| **TCR** | T Cell antigen Receptor |

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