Published in final edited form as:

Microbes Infect. 2010 December ; 12(14-15): 1125–1133. doi:10.1016/j.micinf.2010.08.011.

Natural killer T cells: innate lymphocytes positioned as a bridge between acute and chronic inflammation?

Lisa Fox, Subramanya Hegde, and Jenny E. Gumperz

Department of Medical Microbiology and Immunology, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA

Abstract

Natural killer T cells are an innate population of T lymphocytes that recognize antigens derived from host lipids and glycolipids. In this review, we focus on how these unique T cells are positioned to influence both acute and chronic inflammatory processes through their early recruitment to sites of inflammation, interactions with myeloid antigen presenting cells, and recognition of lipids associated with inflammation.

Keywords

NKT cells; CD1d; Inflammation; Lipid Mediators

1. Introduction

While inflammation has long been recognized as a fundamental set of host responses that are involved in most types of microbial infection, the pathways linking inflammation and antigen-specific immune functions remain poorly understood. Persistent inflammation, a source of much disease pathology, is often associated with the dysregulated activation of antigen-specific lymphocytes, and therefore understanding the processes that link adaptive immunity to inflammatory responses is a question of great importance. In this review, we bring together a series of findings suggesting that a population of innate lymphocytes called Natural Killer T (NKT) cells is uniquely positioned to regulate both the early innate processes of inflammation and the induction of later adaptive responses.

2. A brief overview of inflammation

Inflammation involves a coordinated series of events that can be divided into two parts, called the “acute” and “chronic” phases (see Fig. 1). The acute phase of an inflammatory response is generally initiated by the activation of tissue-resident sentinel cells, such as macrophages, dendritic cells (DCs), and mast cells, as a result of their recognition of danger-associated molecules (e.g. microbial compounds). These cells rapidly release soluble effector molecules, including lipid mediators, vaso-active peptides, cytokines, and chemokines. These soluble factors work together to increase vasodilation and vascular permeability, and to recruit neutrophils and platelets to the site of inflammation. Once in the

Correspondence to: Jenny E. Gumperz.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

tissues, neutrophils assist in clearing the source of the inflammation through phagocytosis of bacteria and dead host cells and through the release of bactericidal agents, while platelets can initiate wound healing.

The chronic phase involves inflammatory processes that are coordinated by T lymphocytes. This phase starts with the trafficking of antigen presenting cells (APCs) to lymphoid tissues, carrying antigens from the site of inflammation (Fig. 1). Within lymphoid tissues, the APCs (i.e. dendritic cells or macrophages) present the inflammation-associated antigens to T cells and provide critical additional stimuli such as co-stimulatory ligands and cytokines. This results in the differentiation and proliferation of antigen-specific effector T cells that leave the lymphoid tissue and infiltrate the site of inflammation. There, they produce effector cytokines such as IFN- γ or IL-17 that both enhance the activation and bacteriocidal effects of phagocytic cells and prolong their inflammatory functions [1].

Acute inflammatory processes are generally self-resolving, in part because this response is mediated by compounds such as bioactive lipids that have short half-lives and are quickly degraded [2], and in part because many of the lipid mediator pathways include compounds that actively inhibit the later stages of inflammation [3]. In contrast, while the chronic phase of inflammatory responses can provide a powerful boost that helps to rapidly clear the infection and resolve the inflammation, if it is not properly controlled this phase can lead to a persistent inflammatory state, characterized by tissue damage mediated by leukocytes and lymphocytes [4].

Perhaps the best examples of persistent inflammatory states that involve antigen-specific chronic inflammation are autoimmune diseases such as type I diabetes and multiple sclerosis. While the role of microbial infection and acute inflammation in the etiology of these diseases remains poorly understood, it is clear that the persistent activation of antigen-specific autopathogenic T cells is central to their disease pathologies. What is also clear is that the chronic inflammatory states that develop in these autoimmune diseases can be counterbalanced by the actions of regulatory T cells that inhibit the effects of autopathogenic T cells. One population of T cells that has been shown to be able to regulate the autoimmune pathology of type I diabetes and multiple sclerosis is the Natural Killer T (NKT) subset (reviewed in [5,6]).

3. NKT cells regulate inflammatory responses

NKT cells are an innate T lymphocyte population that recognizes lipid antigens presented by the non-classical antigen presenting molecule CD1d, which is expressed on myeloid cell types including monocytes, macrophages, and DCs [7,8]. What is remarkable about NKT cells is that they not only can inhibit autoimmune disease by autopathogenic T cells, but they can also promote pro-inflammatory antigen-specific T cell responses, as evidenced by their ability to enhance the reactions of MHC-restricted T cells to protein antigens that were delivered as vaccines [9]. Moreover, NKT cells can enhance the pro-inflammatory functions of other innate lymphocyte subsets such as natural killer (NK) cells [10–12], and they contribute to the clearance of a variety of microbial infections (reviewed in [13–15]). The contrasting immunological effects of NKT cells have been related to their secretion of both Th1 and Th2 cytokines, and, perhaps more importantly, to their ability to interact in varied ways with key APCs (reviewed in [16]). However, what their disparate effects in different immunological contexts also clearly suggest is that NKT cells are best characterized as regulatory cells that contribute to multiple aspects of inflammatory responses.

A number of observations suggest that the role of NKT cells may start during the acute phase of inflammation. These include the finding that most circulating human NKT cells express a pattern of chemokine receptors that is characteristic of T cells that home to sites of

inflammation [17–19]. Moreover, both NKT cells and CD1d⁺ APCs have been found to be enriched at sites of peripheral inflammation, such as periodontal lesions [20,21]. Additionally, we have recently shown that a significant fraction of human NKT cells recognize the presentation by CD1d molecules of a lipid called lyso-phosphatidylcholine (LPC), which is produced by phospholipase A₂ (PLA₂) enzymes during the initiation of inflammatory lipid mediator cascades [22]. Together, these observations suggest that circulating NKT cells may be rapidly recruited to sites of inflammation, and may become specifically activated by CD1d-mediated presentation of lipid antigens that are produced there.

Consistent with this picture, a recent report demonstrates that NKT cells are constituents of the early inflammatory infiltrate in a murine excisional wounding model [23]. The NKT cells were present as early as 12 hours post-wounding, coinciding with the infiltration of neutrophils. Several studies have also indicated that NKT cells may have an impact on neutrophil recruitment or activation during the early stages of bacterial infections. In a murine model of acute pneumonia, CD1d^{-/-} mice, which lack NKT cells, showed poor clearance of *Pseudomonas aeruginosa* [24]. This appeared to be associated with markedly reduced influx of neutrophils into the lungs during the early stages of infection [24], although a different analysis of *P. aeruginosa* infection failed to confirm this neutrophil recruitment defect [25]. However, mice that are specifically deficient in the NKT cell subset did show a defect in neutrophil recruitment into the lungs within the first 12 hours after infection with *Streptococcus pneumoniae* [26], and the neutrophil recruitment defect in this *S. pneumoniae* model could be corrected by adoptive transfer of liver mononuclear cells containing NKT cells but not by transfer of cells from NKT-deficient mice [27]. Moreover, data from this model suggest that production of IFN- γ by NKT cells early in the course of the infection may have a critical role in activating the neutrophil-mediated host defense [27].

Thus, it seems likely that the functions of NKT cells may first come into play during the acute phase and span the transition to chronic inflammatory responses. In the following sections we will first discuss what is known about how NKT cells become activated and interact with myeloid APCs, and will then go on to consider how they may contribute to the cellular events at the site of inflammation.

4. NKT cell activation

Although NKT cells are known for their ability to produce a wide variety of cytokines, they do not necessarily do this in every activation scenario. The specific cytokines produced by NKT cells in any given situation are dependent on a number of factors. A critical component is the level of CD1d expressed by the APC, along with the identity and abundance of the antigens presented. These variables all come together to determine the strength of the T cell receptor (TCR) stimulation experienced by the NKT cell (Fig. 2). We have found that whereas human NKT cells produce a range of cytokines (e.g. GM-CSF, IL-13, IFN- γ , IL-4, IL-2) in response to strong TCR stimulation resulting from exposure to high doses of a high affinity antigen, they produce a more limited set (mainly GM-CSF and IL-13) in response to low levels of TCR stimulation [28]. Additionally, the responses of NKT cells are influenced by cytokines present in the environment (Fig. 2). For example, the presence of IL-12p70 and IL-18 (cytokines that are made by activated myeloid APCs) can compensate for the lack of a strong TCR agonist and drive NKT cells to secrete IFN- γ [28].

Another complexity is the source of antigens that physiologically activate NKT cells. Whereas most peripheral T cells have been subjected to thymic selection ensuring that they have little ability to specifically recognize self antigens, it seems that it is a normal part of the biology of peripheral NKT cells that they are able to specifically recognize certain self

molecules as antigens, as well as recognizing specific microbial lipids (reviewed in [7,14,29]). As a result, NKT cells can be activated by at least two different pathways during infections (Fig. 3). In one route, called the “direct” pathway, it is the recognition of specific microbial lipids that have been ingested and loaded onto CD1d molecules that is thought to stimulate the NKT cell [30–32]. Alternatively, in the “indirect” pathway of NKT cell activation, a foreign lipid need not be present if sufficient pro-inflammatory cytokines are produced by APCs [33–35]. Thus, the direct pathway of NKT cell activation could represent a case in which a strong TCR agonist is present, while the indirect pathway may represent a situation in which TCR stimulation is weak but is compensated by the presence of co-stimulatory cytokines.

The prototypical NKT cell “foreign” antigen is a glycosphingolipid called α -galactosylceramide (α -GalCer). This lipid was originally isolated from non-sterile samples of a marine sponge [36], (thus, it is not clear whether α -GalCer actually derives from the sponge or from bacteria that had colonized it), and has been shown to act as a strong agonist for NKT cells [37]. Mammalian cells do not appear to produce glycosphingolipids of this type in which the sugar is linked in an α -conformation to the lipid, and so α -GalCer is not thought to represent an analogue of self antigens recognized by NKT cells. However, structurally similar glycosphingolipids that are antigenic for NKT cells have been isolated from the cell walls of several *Sphingomonas* bacterial species [30,32,34]. Additionally, diacylglycerol lipids containing an α -linked galactose have been isolated from the spirochete *Borrelia burgdorferi* and been shown to be recognized by NKT cells in a CD1d-dependent manner [31]. Other microbial lipids that may be antigenic for NKT cells include mycobacterial phosphatidylinositol mannosides, lipophosphoglycan from *Leishmania donovani*, and lipopeptidophosphoglycan from *Entamoeba histolytica* [38–40].

What is striking about all of the microbial lipid antigens that have been identified thus far, however, is that none of them seem to be particularly strong TCR agonists for NKT cells, (certainly none are as potent as α -GalCer). Thus, it is not clear that foreign lipids generally do stimulate NKT cells mainly through the direct pathway. Instead, it seems likely that in many cases CD1d-mediated presentation of foreign microbial lipids takes place concurrently with presentation of self lipids that may also be antigenic, and is carried out by APCs that are producing co-stimulatory cytokines such as IL-12 and IL-18 as a result of pattern recognition receptor-induced activation by the microbial compounds that they have ingested (Fig. 3). This scenario is illustrated by a recent analysis of NKT cell activation by lipopeptidophosphoglycan from *E. histolytica*, which found that NKT cell IFN- γ production was dependent both on access to CD1d and on toll like receptor (TLR) stimulated production of IL-12 by the APCs [40]. Thus, since many microbial lipids may constitute only relatively weak TCR agonists for NKT cells, an inflammatory milieu may be required for NKT cell activation by many foreign antigens.

To add another twist to the plot, however, several studies have now come out that point to pathways by which NKT cells may become specifically activated by self antigens that are up-regulated during inflammatory responses. We recently reported that human NKT cells are capable of responding to LPC as an antigen presented by CD1d molecules [22]. LPC is generated by PLA₂-mediated cleavage of membrane phosphatidylcholine (PC) molecules, which is a critical initiating event in the biosynthesis of eicosanoid lipid mediators. Upon exposure of myeloid APCs to inflammatory stimuli, one of the earliest cellular responses is the activation of PLA₂ enzymes [41,42]. The resulting release of arachidonic acid serves as a substrate for the biosynthesis of eicosanoids by two families of enzymes, cyclooxygenases and lipoxygenases, which generate prostanoids and leukotrienes, respectively [2,43]. Eicosanoid lipid mediators such as these are released in large quantities during inflammatory responses and perform critical functions at sites of inflammation, such as influencing local

blood flow and vascular permeability and serving as chemoattractants for neutrophils and monocytes. Interestingly, we found that human peripheral blood monocytes that were pre-treated with an antibody against secreted PLA₂ had unaltered levels of cell surface CD1d, yet were unable to stimulate LPC-reactive NKT cells, suggesting that the antibody treatment blocked their ability to present this self antigen [22]. Thus, based on our data, it is reasonable to speculate that the enzymatic activity of PLA₂ molecules secreted by myeloid APCs at sites of inflammation may lead to increased presentation of LPC by their cell surface CD1d molecules, and that this might enhance the activation of NKT cells, perhaps to the point of inducing cytokines such as IFN- γ and IL-2 that are produced upon exposure to strong TCR signaling (Fig. 4A).

An alternative process by which NKT cell activation may be enhanced during inflammation is suggested by recent findings showing that exposure of myeloid antigen presenting cells to TLR ligands results in changes to cellular glycosphingolipids presented by CD1d molecules and these altered self lipids may provide enhanced stimulation to NKT cells (Fig. 4A). One study found that under physiological conditions, the gangliosides (a type of mammalian glycosphingolipid) bound to murine CD1d molecules were predominantly composed of the species GM1a, GD1a, and GM2. However, upon stimulation with LPS the composition of the lipids bound to CD1d changed, such that GM3 and GD1a were the main species [44]. In another analysis, several of the enzymes involved in the synthesis of cellular glycosphingolipids were up-regulated upon CpG stimulation of APCs, and charged lipids isolated from these APCs appeared to be antigenic for NKT cells [45]. Finally, Salio and colleagues demonstrated that treatment of DCs with a variety of TLR agonists led to increased NKT cell responses that were correlated with increased expression of several enzymes involved in the biosynthesis of glycosphingolipids of the ganglio and globo series [46]. Moreover, fluorescent tetramers made from an NKT cell TCR showed increased binding to TLR-stimulated APCs even though the cell surface CD1d levels were not increased, providing direct evidence to support the hypothesis that a change had taken place that increased the TCR agonism provided by the self lipids bound to the cell surface CD1d molecules. Thus, together these studies suggest that inflammatory processes may increase the abundance or affinity of self antigens presented by CD1d.

Given that NKT cells may be present from quite early on at sites of inflammation, and may be interacting with CD1d⁺ APCs in this context, it seems reasonable to suppose that NKT cells themselves may be targets of lipid mediators (Fig. 4B). This question has only recently started to be explored, however, a recent analysis showed that murine NKT cells express mRNAs for two membrane receptors for prostaglandin D₂ (PGD₂), termed D prostanoid receptor 1 (DP1) and DP2 [47]. Using specific agonists for these receptors, the authors showed that they influence the chemotactic migration of NKT cells, and also demonstrated that exposure to PGD₂ resulted in a selective reduction of NKT cell IFN- γ production while IL-4 secretion was not affected [47]. Whether NKT cell responses are also affected by other lipid mediators that may be produced at sites of acute inflammation is a question that remains to be determined.

5. Modulation of myeloid APC functions

It is now well established that NKT cells are able to interact with a variety of types of myeloid APCs, and can markedly modulate their functional characteristics (recently reviewed in [16]). The major pathways by which NKT cells enhance the pro-inflammatory functions of myeloid APCs include co-stimulation by CD40L, and production of cytokines such as IFN- γ and TNF- α (Figure 5A). In turn, these stimuli cause DCs to mature, up-regulate their own co-stimulatory ligands (e.g. CD80 and CD86), and to maintain long-lasting production of the pro-inflammatory cytokine IL-12p70 [48,49]. It has been clear for

some time that activation of these NKT-mediated pathways of DC stimulation by means of administration of the synthetic glycolipid α -GalCer can have profound effects on adaptive T cell responses to protein antigens [50,51], and more recent studies now suggest that physiologically activated NKT cells may mediate similar effects during microbial infections. For example, it was recently demonstrated in a model of *Chlamydia pneumoniae* infection that the myeloid DCs from mice specifically lacking in NKT cells showed lower expression levels of the maturation markers CD40, CD80, and MHC class II, and also secreted less IL-12 than DCs from NKT cell sufficient mice [52]. Moreover, in a murine model of influenza A infection, NKT cells appeared to relieve the tolerogenic effects of myeloid derived suppressor cells via CD40L co-stimulation, ultimately resulting in enhanced anti-viral immune responses [53]. Thus, NKT cells that interact with myeloid APCs at sites of inflammation may enhance their ability to stimulate adaptive T cell responses after trafficking to secondary lymphoid tissues (see Fig. 1 and Fig. 6).

An intriguing question that remains unresolved, however, is whether NKT cells might also influence the acute phase of inflammatory response via similar pathways (Fig. 5B). For example, several of the cytokines that NKT cells can produce are known to impact lipid mediator production by myeloid APCs. Exposure of monocytes and DCs to IL-4 is reported to increase the expression of the enzyme 15-lipoxygenase and, conversely, to have an inhibitory effect on expression of the enzyme 5-lipoxygenase [54,55]. Both lipoxygenase enzymes metabolize arachidonic acid, but the outcome is different: 5-lipoxygenase activity leads to the biosynthesis of leukotriene B₄, a chemoattractant for neutrophils, whereas 15-lipoxygenase activity leads to the generation of lipoxins, which help mediate the resolution of inflammation. Interestingly, exposure to IL-13 might enhance the effects of IL-4, since this cytokine has been shown to increase the expression of cytoplasmic forms of PLA₂, possibly leading to the generation of more arachidonic acid substrate for eicosanoid biosynthesis [56]. Additionally, IL-13 treatment has been shown to inhibit the induction of cyclooxygenase-2 enzymes, thus limiting prostaglandin production, which could have the effect of enhancing the biosynthesis of leukotrienes by lipoxygenases [57]. Ligation of CD40 on human monocytes has been shown to result in ERK and p38 MAPK signaling as well as in activation of the transcription factor NF κ B, and to result in increased synthesis of PGE₂ by cyclooxygenase-2 enzymes, although this effect was modulated by exposure to IL-4 [58]. Thus, it may be quite reasonable to suppose that NKT cells directly regulate acute inflammatory responses by exerting effects on the lipid mediator production of myeloid APCs at sites of inflammation.

6. Concluding remarks

Although NKT cells comprise a comparatively small fraction of the total T lymphocyte population, they are now clearly recognized as a potent immunoregulatory population. Their outsized ability to influence immunological outcomes has been attributed to their rapid cytokine production and to their ability to modulate the functions of critical APCs. In this review, we bring together results that suggest an additional reason for the far-reaching effects of NKT cells: as a result of their early recruitment to sites of inflammation and recognition of lipid antigens produced during inflammation, they may be uniquely positioned to interact with key APCs in a way that spans the acute and chronic phases of inflammation (Fig. 6). This picture of NKT cells raises a number of interesting questions for future research, including understanding their influence on lipid mediator production by myeloid APCs and, conversely, determining how the functions of NKT cells are influenced by lipid mediators produced at sites of inflammation. Also of interest, do NKT cells interact directly with other key cell types found at sites of inflammations, such as mast cells, neutrophils, platelets? Finally, a full understanding of the role of NKT cells in inflammatory contexts will involve assessing the significance of other, related T cell populations that may

recognize similar lipid antigens. For example, a recent report demonstrated that LPC-specific CD1d-restricted T cells that appeared phenotypically and functionally distinct from NKT cells are expanded in the blood of many multiple myeloma patients [59]. Thus, the normal regulatory functions of NKT cells during inflammation may be overcome in certain pathological conditions by other CD1d-restricted T cells that have a more harmful impact.

References

1. Muller I, Munder M, Kropf P, Hansch GM. Polymorphonuclear neutrophils and T lymphocytes: strange bedfellows or brothers in arms? *Trends Immunol* 2009;30:522–530. [PubMed: 19775938]
2. Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* 2001;294:1871–1875. [PubMed: 11729303]
3. Serhan CN, Savill J. Resolution of inflammation: the beginning programs the end. *Nat Immunol* 2005;6:1191–1197. [PubMed: 16369558]
4. Nathan C, Ding A. Nonresolving inflammation. *Cell* 140:871–882. [PubMed: 20303877]
5. Cardell SL. The natural killer T lymphocyte: a player in the complex regulation of autoimmune diabetes in non-obese diabetic mice. *Clin Exp Immunol* 2006;143:194–202. [PubMed: 16412042]
6. Wu L, Van Kaer L. Natural killer T cells and autoimmune disease. *Curr Mol Med* 2009;9:4–14. [PubMed: 19199937]
7. Bendelac A, Savage PB, Teyton L. The biology of NKT cells. *Annu Rev Immunol* 2007;25:297–336. [PubMed: 17150027]
8. Brigl M, Brenner MB. CD1: Antigen Presentation and T Cell Function. *Annu Rev Immunol* 2004;22:817–890. [PubMed: 15032598]
9. Cerundolo V, Silk JD, Masri SH, Salio M. Harnessing invariant NKT cells in vaccination strategies. *Nat Rev Immunol* 2009;9:28–38. [PubMed: 19079136]
10. Carnaud C, Lee D, Donnars O, Park SH, Beavis A, Koezuka Y, Bendelac A. Cutting edge: Cross-talk between cells of the innate immune system: NKT cells rapidly activate NK cells. *J Immunol* 1999;163:4647–4650. [PubMed: 10528160]
11. Eberl G, MacDonald HR. Selective induction of NK cell proliferation and cytotoxicity by activated NKT cells. *Eur J Immunol* 2000;30:985–992. [PubMed: 10760785]
12. Smyth MJ, Crowe NY, Pellicci DG, Kyriakoudis K, Kelly JM, Takeda K, Yagita H, Godfrey DI. Sequential production of interferon-gamma by NK1.1(+) T cells and natural killer cells is essential for the antitumorigenic effect of alpha-galactosylceramide. *Blood* 2002;99:1259–1266. [PubMed: 11830474]
13. Skold M, Behar SM. Role of CD1d-restricted NKT cells in microbial immunity. *Infect Immun* 2003;71:5447–5455. [PubMed: 14500461]
14. Cohen NR, Garg S, Brenner MB. Antigen Presentation by CD1 Lipids, T Cells, and NKT Cells in Microbial Immunity. *Adv Immunol* 2009;102:1–94. [PubMed: 19477319]
15. Kronenberg M, Kinjo Y. Innate-like recognition of microbes by invariant natural killer T cells. *Curr Opin Immunol* 2009;21:391–396. [PubMed: 19646850]
16. Hegde S, Fox L, Wang X, Gumperz JE. Autoreactive natural killer T cells: promoting immune protection and immune tolerance through varied interactions with myeloid antigen-presenting cells. *Immunology* 2010;130:471–483. [PubMed: 20465577]
17. Gumperz JE, Miyake S, Yamamura T, Brenner MB. Functionally Distinct Subsets of CD1d-restricted Natural Killer T Cells Revealed by CD1d Tetramer Staining. *J Exp Med* 2002;195:625–636. [PubMed: 11877485]
18. Kim CH, Johnston B, Butcher EC. Trafficking machinery of NKT cells: shared and differential chemokine receptor expression among V alpha 24(+)V beta 11(+) NKT cell subsets with distinct cytokine-producing capacity. *Blood* 2002;100:11–16. [PubMed: 12070001]
19. Thomas SY, Hou R, Boyson JE, Means TK, Hess C, Olson DP, Strominger JL, Brenner MB, Gumperz JE, Wilson SB, Luster AD. CD1d-restricted NKT cells express a chemokine receptor profile indicative of Th1-type inflammatory homing cells. *J Immunol* 2003;171:2571–2580. [PubMed: 12928408]

20. Yamazaki K, Ohsawa Y, Yoshie H. Elevated proportion of natural killer T cells in periodontitis lesions: a common feature of chronic inflammatory diseases. *Am J Pathol* 2001;158:1391–1398. [PubMed: 11290557]
21. Amanuma R, Nakajima T, Yoshie H, Yamazaki K. Increased infiltration of CD1d and natural killer T cells in periodontal disease tissues. *J Periodontal Res* 2006;41:73–79. [PubMed: 16409258]
22. Fox LM, Cox DG, Lockridge JL, Wang X, Chen X, Scharf L, Trott DL, Ndonge RM, Veerapen N, Besra GS, Howell AR, Cook ME, Adams EJ, Hildebrand WH, Gumperz JE. Recognition of lyso-phospholipids by human natural killer T lymphocytes. *PLoS Biol* 2009;7:e1000228. [PubMed: 19859526]
23. Schneider DF, Palmer JL, Tulley JM, Speicher JT, Kovacs EJ, Gamelli RL, Faunce DE. A Novel Role for NKT Cells in Cutaneous Wound Repair. *J Surg Res*. 2009
24. Nieuwenhuis EE, Matsumoto T, Exley M, Schleipman RA, Glickman J, Bailey DT, Corazza N, Colgan SP, Onderdonk AB, Blumberg RS. CD1d-dependent macrophage-mediated clearance of *Pseudomonas aeruginosa* from lung. *Nat Med* 2002;8:588–593. [PubMed: 12042809]
25. Kinjo T, Nakamatsu M, Nakasone C, Yamamoto N, Kinjo Y, Miyagi K, Uezu K, Nakamura K, Higa F, Tateyama M, Takeda K, Nakayama T, Taniguchi M, Kaku M, Fujita J, Kawakami K. NKT cells play a limited role in the neutrophilic inflammatory responses and host defense to pulmonary infection with *Pseudomonas aeruginosa*. *Microbes Infect* 2006;8:2679–2685. [PubMed: 16979364]
26. Kawakami K, Yamamoto N, Kinjo Y, Miyagi K, Nakasone C, Uezu K, Kinjo T, Nakayama T, Taniguchi M, Saito A. Critical role of Valpha14+ natural killer T cells in the innate phase of host protection against *Streptococcus pneumoniae* infection. *Eur J Immunol* 2003;33:3322–3330. [PubMed: 14635040]
27. Nakamatsu M, Yamamoto N, Hatta M, Nakasone C, Kinjo T, Miyagi K, Uezu K, Nakamura K, Nakayama T, Taniguchi M, Iwakura Y, Kaku M, Fujita J, Kawakami K. Role of interferon-gamma in Valpha14+ natural killer T cell-mediated host defense against *Streptococcus pneumoniae* infection in murine lungs. *Microbes Infect* 2007;9:364–374. [PubMed: 17314060]
28. Wang X, Chen X, Rodenkirch L, Simonson W, Wernimont S, Ndonge RM, Veerapen N, Gibson D, Howell AR, Besra GS, Painter GF, Huttenlocher A, Gumperz JE. Natural killer T-cell autoreactivity leads to a specialized activation state. *Blood* 2008;112:4128–4138. [PubMed: 18779390]
29. Zajonc DM, Kronenberg M. Carbohydrate specificity of the recognition of diverse glycolipids by natural killer T cells. *Immunol Rev* 2009;230:188–200. [PubMed: 19594637]
30. Kinjo Y, Wu D, Kim G, Xing GW, Poles MA, Ho DD, Tsuji M, Kawahara K, Wong CH, Kronenberg M. Recognition of bacterial glycosphingolipids by natural killer T cells. *Nature* 2005;434:520–525. [PubMed: 15791257]
31. Kinjo Y, Tupin E, Wu D, Fujio M, Garcia-Navarro R, Benhnia MR, Zajonc DM, Ben-Menachem G, Ainge GD, Painter GF, Khurana A, Hoebe K, Behar SM, Beutler B, Wilson IA, Tsuji M, Sellati TJ, Wong CH, Kronenberg M. Natural killer T cells recognize diacylglycerol antigens from pathogenic bacteria. *Nat Immunol* 2006;7:978–986. [PubMed: 16921381]
32. Sriram V, Du W, Gervay-Hague J, Brutkiewicz RR. Cell wall glycosphingolipids of *Sphingomonas paucimobilis* are CD1d-specific ligands for NKT cells. *Eur J Immunol* 2005;35:1692–1701. [PubMed: 15915536]
33. 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. [PubMed: 14578883]
34. Mattner J, Debord KL, Ismail N, Goff RD, Cantu C 3rd, Zhou D, Saint-Mezard P, Wang V, Gao Y, Yin N, Hoebe K, Schneewind O, Walker D, Beutler B, Teyton L, Savage PB, Bendelac A. Exogenous and endogenous glycolipid antigens activate NKT cells during microbial infections. *Nature* 2005;434:525–529. [PubMed: 15791258]
35. Nagarajan NA, Kronenberg M. Invariant NKT cells amplify the innate immune response to lipopolysaccharide. *J Immunol* 2007;178:2706–2713. [PubMed: 17312112]

36. Kawano T, Cui J, Koezuka Y, Toura I, Kaneko Y, Motoki K, Ueno H, Nakagawa R, Sato H, Kondo E, Koseki H, Taniguchi M. CD1d-restricted and TCR-mediated activation of valpha14 NKT cells by glycosylceramides. *Science* 1997;278:1626–1629. [PubMed: 9374463]
37. Crowe NY, Uldrich AP, Kyparissoudis K, Hammond KJ, Hayakawa Y, Sidobre S, Keating R, Kronenberg M, Smyth MJ, Godfrey DI. Glycolipid antigen drives rapid expansion and sustained cytokine production by NK T cells. *J Immunol* 2003;171:4020–4027. [PubMed: 14530322]
38. Fischer K, Scotet E, Niemeyer M, Koebernick H, Zerrahn J, Maillet S, Hurwitz R, Kursar M, Bonneville M, Kaufmann SH, Schaible UE. Mycobacterial phosphatidylinositol mannoside is a natural antigen for CD1d-restricted T cells. *Proc Natl Acad Sci U S A* 2004;101:10685–10690. [PubMed: 15243159]
39. Amprey JL, Im JS, Turco SJ, Murray HW, Illarionov PA, Besra GS, Porcelli SA, Spath GF. A subset of liver NK T cells is activated during *Leishmania donovani* infection by CD1d-bound lipophosphoglycan. *J Exp Med* 2004;200:895–904. [PubMed: 15466622]
40. Lotter H, Gonzalez-Roldan N, Lindner B, Winau F, Isibasi A, Moreno-Lafont M, Ulmer AJ, Holst O, Tannich E, Jacobs T. Natural killer T cells activated by a lipopeptidophosphoglycan from *Entamoeba histolytica* are critically important to control amebic liver abscess. *PLoS Pathog* 2009;5:e1000434. [PubMed: 19436711]
41. Exton JH. Phosphatidylcholine breakdown and signal transduction. *Biochim Biophys Acta* 1994;1212:26–42. [PubMed: 8155724]
42. Touqui L, Alaoui-El-Azher M. Mammalian secreted phospholipases A2 and their pathophysiological significance in inflammatory diseases. *Curr Mol Med* 2001;1:739–754. [PubMed: 11899260]
43. Gronert K. Lipid autacoids in inflammation and injury responses: a matter of privilege. *Mol Interv* 2008;8:28–35. [PubMed: 18332482]
44. Muindi K, Cernadas M, Watts GF, Royle L, Neville DC, Dwek RA, Besra GS, Rudd PM, Butters TD, Brenner MB. Activation state and intracellular trafficking contribute to the repertoire of endogenous glycosphingolipids presented by CD1d [corrected]. *Proc Natl Acad Sci U S A* 2010;107:3052–3057. [PubMed: 20133624]
45. Paget C, Mallevaey T, Speak AO, Torres D, Fontaine J, Sheehan KC, Capron M, Ryffel B, Faveeuw C, Leite de Moraes M, Platt F, Trottein F. 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. [PubMed: 17950005]
46. Salio M, Speak AO, Shepherd D, Polzella P, Illarionov PA, Veerapen N, Besra GS, Platt FM, Cerundolo V. Modulation of human natural killer T cell ligands on TLR-mediated antigen-presenting cell activation. *Proc Natl Acad Sci U S A* 2007;104:20490–20495. [PubMed: 18077358]
47. Torres D, Paget C, Fontaine J, Mallevaey T, Matsuoka T, Maruyama T, Narumiya S, Capron M, Gosset P, Faveeuw C, Trottein F. Prostaglandin D2 inhibits the production of IFN-gamma by invariant NK T cells: consequences in the control of B16 melanoma. *J Immunol* 2008;180:783–792. [PubMed: 18178816]
48. Kitamura H, Iwakabe K, Yahata T, Nishimura S, Ohta A, Ohmi Y, Sato M, Takeda K, Okumura K, Van Kaer L, Kawano T, Taniguchi M, Nishimura T. The natural killer T (NKT) cell ligand alpha-galactosylceramide demonstrates its immunopotentiating effect by inducing interleukin (IL)-12 production by dendritic cells and IL-12 receptor expression on NKT cells. *J Exp Med* 1999;189:1121–1128. [PubMed: 10190903]
49. Vincent MS, Leslie DS, Gumperz JE, Xiong X, Grant EP, Brenner MB. CD1-dependent dendritic cell instruction. *Nat Immunol* 2002;3:1163–1168. [PubMed: 12415264]
50. Fujii S, Liu K, Smith C, Bonito AJ, Steinman RM. The linkage of innate to adaptive immunity via maturing dendritic cells in vivo requires CD40 ligation in addition to antigen presentation and CD80/86 costimulation. *J Exp Med* 2004;199:1607–1618. [PubMed: 15197224]
51. Fujii S, Shimizu K, Smith C, Bonifaz L, Steinman RM. Activation of natural killer T cells by alpha-galactosylceramide rapidly induces the full maturation of dendritic cells in vivo and thereby acts as an adjuvant for combined CD4 and CD8 T cell immunity to a coadministered protein. *J Exp Med* 2003;198:267–279. [PubMed: 12874260]

52. Joyee AG, Qiu H, Fan Y, Wang S, Yang X. Natural killer T cells are critical for dendritic cells to induce immunity in Chlamydial pneumonia. *Am J Respir Crit Care Med* 2008;178:745–756. [PubMed: 18596223]
53. De Santo C, Salio M, Masri SH, Lee LY, Dong T, Speak AO, Porubsky S, Booth S, Veerapen N, Besra GS, Grone HJ, Platt FM, Zambon M, Cerundolo V. Invariant NKT cells reduce the immunosuppressive activity of influenza A virus-induced myeloid-derived suppressor cells in mice and humans. *J Clin Invest* 2008;118:4036–4048. [PubMed: 19033672]
54. Profita M, Sala A, Siena L, Henson PM, Murphy RC, Paterno A, Bonanno A, Riccobono L, Mirabella A, Bonsignore G, Vignola AM. Leukotriene B4 production in human mononuclear phagocytes is modulated by interleukin-4-induced 15-lipoxygenase. *J Pharmacol Exp Ther* 2002;300:868–875. [PubMed: 11861792]
55. Spanbroek R, Hildner M, Kohler A, Muller A, Zintl F, Kuhn H, Radmark O, Samuelsson B, Habenicht AJ. IL-4 determines eicosanoid formation in dendritic cells by down-regulation of 5-lipoxygenase and up-regulation of 15-lipoxygenase 1 expression. *Proc Natl Acad Sci U S A* 2001;98:5152–5157. [PubMed: 11320251]
56. Rey A, M'Rini C, Sozzani P, Lamboeuf Y, Beraud M, Caput D, Ferrara P, Pipy B. IL-13 increases the cPLA2 gene and protein expression and the mobilization of arachidonic acid during an inflammatory process in mouse peritoneal macrophages. *Biochim Biophys Acta* 1998;1393:244–252. [PubMed: 9748607]
57. Endo T, Ogushi F, Sone S. LPS-dependent cyclooxygenase-2 induction in human monocytes is down-regulated by IL-13, but not by IFN-gamma. *J Immunol* 1996;156:2240–2246. [PubMed: 8690914]
58. Inoue Y, Otsuka T, Niino H, Nagano S, Arinobu Y, Ogami E, Akahoshi M, Miyake K, Ninomiya I, Shimizu S, Nakashima H, Harada M. Novel regulatory mechanisms of CD40-induced prostanoid synthesis by IL-4 and IL-10 in human monocytes. *J Immunol* 2004;172:2147–2154. [PubMed: 14764680]
59. Chang DH, Deng H, Matthews P, Krasovsky J, Ragupathi G, Spisek R, Mazumder A, Vesole DH, Jagannath S, Dhodapkar MV. Inflammation-associated lysophospholipids as ligands for CD1d-restricted T cells in human cancer. *Blood* 2008;112:1308–1316. [PubMed: 18535199]

Fig. 1. Overview of inflammation

Upon wounding or a bacterial infection, tissue-resident myeloid cells (macrophages and DCs) and mast cells become activated and produce a variety of cytokines, chemokines, vasoactive peptides, and lipid mediators. This results in vasodilation, enhanced vascular permeability, and the activation of platelets. These molecules also recruit neutrophils into the tissues, where they become activated. This constitutes the acute phase of inflammation. The initiation of the chronic phase of inflammation begins when myeloid APCs migrate to secondary lymphoid tissues, where they present antigens to T lymphocytes and provide critical additional signals (e.g. expression of co-stimulatory molecules and production of cytokines). Antigen-specific T lymphocytes become activated and migrate to the source of inflammation, where they secrete cytokines that enhance to phagocytic and bacteriocidal

properties of macrophages, neutrophils, and other cells. The continuous activation of neutrophils prevents them from undergoing apoptosis, and can lead to a persistent inflammatory state.

Fig. 2. Determinants of cytokine output

Although NKT cells are capable of producing a wide variety of cytokines [17], the specific cytokines produced in any given situation depend on a number of different variables. These include: the level of CD1d expressed by the APC (1) and the type and quantity of antigen presented (2). These factors determine the strength of the signal received through the TCR (3), which in turn directs cytokine output: GM-CSF and IL-13 require less TCR stimulation, with IL-4, IFN γ , and IL-2 production requiring a stronger stimulus [28]. The presence of co-stimulating cytokines such as IL-12 and IL-18 (4) can amplify NKT cell responses to weak or rare antigens, and stimulate the production of IFN γ [28].

Fig. 3. Activation by lipid antigens

NKT cells can be activated to produce IFN- γ either by “direct” recognition of foreign microbial antigens, or “indirectly” by recognition of self lipids that are presented along with co-stimulating cytokines produced by myeloid APCs that have received stimulation through a pattern recognition receptor (PRR), such as one of the toll-like receptors (TLR). Notably, these pathways are not mutually exclusive. In many cases, lipid antigen uptake by APCs resulting in presentation by the direct pathway may involve TLR-stimulation, and cytokine production by the APCs. Moreover, foreign antigens probably do not displace all of the self-antigens presented by the CD1d molecules on an APC, and thus NKT cells may continue to be stimulated by recognition of self-antigens despite the presence of foreign microbial compounds.

Fig. 4. Effects of inflammatory lipids

A) NKT cells recognize specific lipids that are produced during inflammation as antigens presented by CD1d molecules. Microbial stimulation of myeloid antigen presenting cells results in the activation of secreted PLA₂ enzymes. These cleave phosphatidylcholine molecules in lipid membranes, releasing lyso-phosphatidylcholine (LPC). LPC can bind to CD1d molecules, and is recognized as an antigen by human NKT cells [22]. Additionally, microbial stimulation of myeloid APCs alters the types of glycosphingolipids presented by CD1d molecules, and this may result in increased TCR stimulation of NKT cells [44–46]. **B)** Because of their early recruitment to sites of inflammation, NKT cells may be targets for lipid mediators produced by APCs. NKT cells have been shown to express the PGD₂ receptors DP1 and DP2, and to respond to stimulation through these receptors by altering their migration and selectively reducing secretion of IFN-γ but not IL-4 [47].

Fig. 5. Modulation of myeloid APCs

A) Upon recognition of antigen presented by CD1d, NKT cells up-regulate their cell surface expression of CD40L, and produce cytokines such as $\text{IFN-}\gamma$ and $\text{TNF-}\alpha$. Stimulation of myeloid APCs through ligation of CD40 and recognition of these cytokines results in increased expression of co-stimulatory molecules (e.g. CD80 and CD86) and altered production of cytokines such as IL-10 and IL-12. These APC changes can have a profound effect on their ability to subsequently stimulate antigen-specific MHC-restricted T cells [48,50,51]. **B)** Similar pathways of NKT cell-mediated co-stimulation may influence the types of lipid mediators produced by myeloid APCs. The cytokines IL-4 and IL-13, which can be produced by NKT cells upon recognition of antigens presented by CD1d, have been reported to impact expression of lipoxygenase enzymes involved in the generation of lipid mediators [54,55]. Additionally, stimulation of myeloid APCs through CD40 induces activation of NF κ B and MAPK signaling processes that can influence expression of cyclooxygenase-2 enzymes resulting in altered prostaglandin production [58].

Fig. 6. NKT cells influence both the acute and chronic branches of inflammation

By interacting with myeloid APCs at sites of inflammation, NKT cells may be particularly well positioned to influence both the acute and chronic phases of inflammatory responses. Current data on the immunological effects of NKT cells support this model, as they have been shown to impact the recruitment and/or activation of neutrophils as well as the induction of antigen-specific T cell responses during microbial infections.