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Natural Killer T Lymphocytes Integrate Innate Sensory Information and Relay Context to Effector Immune Responses

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Abstract

It is now appreciated that a group of lymphoid lineage cells, collectively called innate-like effector lymphocytes, have evolved to integrate information relayed by the innate sensory immune system about the state of the local tissue environment and to pass on this context to downstream effector innate and adaptive immune responses. Thereby, innate functions engrained into such innate-like lymphoid lineage cells during development can control the quality and magnitude of an immune response to a tissue-altering pathogen and facilitate the formation of memory engrams within the immune system. These goals are accomplished by the innate lymphoid cells that lack antigen-specific receptors, γδ T cell receptor (TCR)-expressing T cells, and several αβ TCR-expressing T cell subsets—such as natural killer T cells, mucosal-associated invariant T cells, et cetera. Whilst we briefly consider the commonalities in the origins and functions of these diverse lymphoid subsets to provide context, the primary topic of this review is to discuss how the semi-invariant natural killer T cells got this way in evolution through lineage commitment and onward ontogeny. What emerges from this discourse is the question: Has a “limbic immune system” emerged (screaming quietly in plain sight!) out of what has been dubbed “in-betweeners”?

Keywords

NKT cells; function; evolution; development

I. INTRODUCTION

The immune system is one constituent of the 10 physiologic systems of the body. It consists of two arms: the innate and adaptive immune systems. These two immune systems work in concert to sense alteration/s in the internal milieu—Claude Bernard’s *milieu de l’intérieur* or Walter Cannon’s homeostasis (see origins in[1–3])—to process and integrate the perceived

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information, and actuate a response tailored to the altered state/s. Usually, the quick-to-act innate immune response is sufficient to return the host's altered internal milieu back to normalcy; when it fails, however, the adaptive immune system is fully engaged. Even when the innate immune response is sufficient to ward off inflammatory intruders, an adaptive immune response is initiated so that memory engrams of the first encounter can be established in anticipation of future engagements. One end-product of the innate immune response is the display of fragments—breakdown products of chemical constituents derived from agents that incite alterations in the host's internal milieu—on the surface of certain innate immune cells called dendritic cells (DCs). T lymphocytes recognize such fragmentary end-products—largely peptides, but also lipids, vitamin metabolites, and xenobiotics—to initiate an adaptive immune response. Whilst this initial recognition imparts specificity to the reaction, T lymphocytes require additional signals to elaborate a context-dependent response. This context is established by the nature of soluble mediators—cytokines and chemokines—secreted by the activated innate immune cells and the innate-like effector lymphocytes (ILELs).

The innate immune response occurs locally at barrier sites, such as the oral–respiratory, gastrointestinal, and urogenital mucosae, and the skin—the common ports of entry of noxious substances and microorganisms. The primary adaptive T cell response occurs in the local lymph nodes draining the various tissues of the host. The topologic barrier so imposed is solved by the migration of activated resident DCs from the barrier site to the local draining lymph node wherein processed antigens are presented to naïve, antigen-specific T cells. This DC-T cell interaction initiates an adaptive immune response, connecting innate immunity to the adaptive immune responses.

ILELs have evolved in vertebrates to bridge the innate and adaptive immune responses (Fig. 1). ILELs are a group of unconventional lymphoid lineage cells that either do not express antigen-specific receptors (innate lymphoid cells, ILCs) or express a defined repertoire of antigen-specific receptors generated through somatic recombination (innate-like T and B lymphocytes). Unlike conventional T and B cells, unconventional T & B lymphocytes exhibit innate-like recognition and functional characteristics (Fig. 2).[4–7] Innate-like lymphocytes include both T [γδT and αβT cells such as natural killer T cells (NKT), mucosal-associated invariant T (MAIT) lymphocytes, and CD8αα-expressing intestinal intraepithelial lymphocytes] and B cells (B-1 and marginal zone B cells). Importantly, ILCs and innate-like T cells, like conventional T cells mediate type 1, type 2, and type 3 immunity (Fig. 2). As such, this group of immune cells can initiate, amplify, and fine-tune both the innate and adaptive immune responses.[5,7,8] Engagement of multiple immune modules result in a context-dependent inflammatory response to maintain a stable *milieu intérieur*.[9]

NKT cells constitute one group of thymus-derived ILELs. NKT cell functions are controlled by CD1d restricted self and non-self lipid agonists.[10] The majority of NKT cells (type I, invariant NKT cells—the protagonist of this review) express an invariant TCR α-chain generated by TRAV11*02 (mouse Vα14i) or TRAV10 (human Vα24i) to TRAJ18 (Jα18) rearrangement. The invariant α-chain largely pairs with mouse TRBV13–2*01 (Vβ8.2), TRBV29*02 (Vβ7), TRBV1 (Vβ2), or human TRBV25–1 (Vβ11) β-chain to form a functional semi-invariant TCR. At least in mice, a smaller NKT cell population—the

type II NKT cells (reviewed elsewhere)—expresses a more diverse TCR repertoire and recognizes CD1d-restricted lipid antigens.[11–16] Activated NKT cells rapidly secrete a variety of cytokines and chemokines, and upregulate costimulatory molecules. Similarly, MAIT cells and γδT cells also express a restricted TCR repertoire (Fig. 2).[5,7] The ability of ILELs to respond quickly to specific agonists allows them to alert and steer downstream effector functions of myeloid and lymphoid cells. Hence, ILELs can control immunity to microbes and cancers, as well as autoimmune and inflammatory diseases.[5,7,8,17–19]

II. NKT CELL ACTIVATION AND FUNCTIONS—PARALLELS IN OTHER INNATE-LIKE EFFECTOR LYMPHOCYTES

> *"Nil ideo quoniam natumst in corpore ut uti possemus, sed quod natumst id procreat usum"* (translated: "For nothing is born in the body in order that we may use it, but rather, having been born, it begets a use"[20,21]).

CD1d molecules, which bind to and present a variety of lipid ligands (Table 1 and references therein), control NKT cell functions.[22] Pioneering studies from several research groups have shed light on how infectious agents or derived compounds activate NKT cells. Early studies demonstrated that DCs—by either directly presenting a microbial lipid/s or indirectly by inducing a self-lipid/s upon pathogen recognition—play an important role in NKT cell activation.[22–25] Bacteria and certain fungi that biosynthesize glycosphinsphingolipids (GSLs) or glycoglycerolipids (GGLs), which when presented at the surface of DCs by CD1d, activate NKT cells.[26–34] But, just as interestingly, microbes—both bacteria and viruses—that do not make NKT cell agonist/s themselves also activate NKT cells. This requires the expression of a pathogen-associated molecular pattern (PAMP) by the microbe and a PRR (pattern recognition receptor) by DCs.[22,24,25] How both categories of microbes activate NKT cells is described below.

Our current understanding of NKT cell functions was gleaned from numerous *in vitro* and *in vivo* studies using the GSL, α-galactosylceramide (αGalCer, KRN7000), and its analogues (Table 1, and references therein). αGalCer/KRN7000—a potent NKT cell agonist with anti-cancer properties—is a natural compound isolated from the marine sponge, *Agelas mauritianus*, likely derived from its symbionts (Table 1, and references therein). NKT cell biology so gleaned came to question because marine sponges and associated symbionts are neither vertebrate parasites nor pathogens. Notwithstanding that, few *Sphingomonas* species, *Bacteroides fragilis*, *Aspergillus fumigatus*, and mammalian cells also generate αGalCers and/or related compounds (Table 1, and references therein). The prevalence of αGalCer and related compounds in nature lends credence to NKT cell biology learned from studies with αGalCer/KRN7000.

A significant advance in MAIT cell biology was the discovery that the restricting MHC-related 1 (MR1) assembled with small molecules such as the folic acid (vitamin B9) metabolite 6-formylpterin (6-FP), a photodegradation product of B9. Although eluted from MR1, 6-FP is not a MAIT cell agonist.[35] Further experiments revealed that bacterial species containing the yet to be identified *rib* gene for enzymatic production of the riboflavin biosynthetic intermediate 5-amino-6-d-ribitylaminouracil (5-A-RU),[36] generated a MAIT

cell agonist/s, whereas those bacterial species lacking this *rib* gene did not.[35] It was then found that the vitamin B metabolite 5-(2-oxopropylideneamino)-6-d-ribitylaminouracil (5-OP-RU) activated MAIT cells when presented by MR1 molecules.[35] Much of what we know about MAIT cells owes to this seminal discovery (reviewed in refs. [5,7,37,38]).

Some mouse and human γδT cells are CD1d-restricted. They recognize the GPL cardiolipin, or the GSLs αGalCer or sulfatide.[39–42] Some mouse γδT cell clones recognize either nonclassical MHC class Ib molecules—such as the ligand-free T10 and T22[43–48]—or MHC class II molecules without peptide specificity.[49,50] The specialized mouse dendritic epidermal T cells express Vγ9–Vδ2 TCR, home to the skin, and recognize Skint-1 expressed by keratinocytes.[46,48,51,52] Most human γδT cells express the Vγ9–Vδ2 TCR and are specific for self or bacterial alkylamines, amino-bis-phosphonates, and phosphoantigens, which are presented by butyrophilins in a highly unconventional manner independent of a conventional antigen-binding groove as seen in MHC and related molecules.[46,48,51] Other human γδT cells recognize stress-induced MIC, ULBP4, or endothelial protein C receptor.[53] The recognition principles of some but not all agonistic γδTCRs have been elucidated.[7]

A. Multiple Mechanisms Activate NKT Cells: Parallels with Other Innate Effector Cells

1. Agonist-Dominated NKT Cell Activation—αGalCer, when presented by CD1d molecules, activates NKT cells in a TCR-dependent manner. Likewise, *B. fragilis*–derived αGalCer also directly activates NKT cells (Table 1, and references therein). Such an activation mechanism is considered agonist-dominated activation (Fig. 3, left panel).

2. Agonist-Dominated MAIT Cell Activation—MAIT cells share with NKT cells an agonist-dominated activation mechanism. As discussed above, MAIT cells recognize a limited number of agonistic small molecules. The best studied is the vitamin B metabolite 5-OP-RU.[7,37,54] This agonist is biosynthesized by several Gram-positive and Gram-negative bacterial species that carry genes coding for enzymes that calalyze reactions in the riboflavin A biosynthetic pathway (reviewed in ref. [37]).

3. Agonist- and Cytokine-Dominated NKT Cell Activation—Certain bacterial species generate weak NKT cell agonists; for example, *Aspergilus fumigatus* and *Sphingomonas* spp. biosynthesize asparamide B and α-galacturonosylceramide (αGalACer; Table 1; see references therein), respectively, which are αGalCer-related GSLs. Certain bacteria synthesise GGLs—e.g., α-galactosyldiacylglycerol by *Borrelia burgdorferi* and α-glucosyldiacylglycerol by *Streptococcus pneumoniae*—and cholesteryl-α-glycoside such cholesteryl-6-O-acyl α-glucoside by *Helicobacter pylori* (Table 1; see references therein). In the case of these weak agonists, NKT cell activation requires a second signal. Inflammatory cytokine/s elicited by DCs activated through their PRRs[24,25,55] serve as a second signal. This form of NKT cell activation is considered agonist- and cytokine-dominated activation (Fig. 3, middle panel).

In many instances, the activation of antigen-presenting cells (APCs) by bacteria, fungi, and viruses induces self lipid biosynthesis and the generation of NKT cell self lipid agonists. Such self-agonists include glycerophospholipids (GPLs), mammalian αGalCer, and the GSL, iGb3 (isoglobotrihexosylceramide).[31,56–59] NKT cells respond to CD1d

molecules presenting self-lipids on host APCs in the presence of an inflammatory second signal.[8,60] Notably, the inability of βGlcCer synthase-deficient cells to activate autoreactive NKT cell hybridomas[61] and βGlcCer synthase-deficient thymocytes to support NKT cell development[62] pointed to a cellular βGlcCer or a derived GSL as an endogenous mouse NKT cell agonist.[61,62]

As self-lipids are weak agonists, NKT cell activation requires a second signal such as IL-12 elicited upon activation via PRRs such as dectin-1[26,55] or toll-like receptor (TLR)-4.[24,25] Type I interferon (IFN-I) produced by CpG-TLR9-activated DCs is also known to serve as a second signal for NKT cell activation by sialylated cellular glycolipids presented by CD1d molecules.[63] Hence, this mode of activation is a variation of the agonist- and cytokine-dominated activation (Fig. 3, middle panel). Almost all virus infections and certain bacterial infections induce an IFN-I response.[64–75] Hence, NKT cell activation by a self-agonist in conjunction with IFN-I notifies the host of a microbial infection even when an invading pathogen does not biosynthesize an NKT cell agonist.

4. **Agonist- and Cytokine-Dominated MAIT Cell Activation—**As virus and bacterial infections elicit innate cytokine responses, one study examined whether type I IFNs influence MAIT cell activation even when an agonist is presented. Akin to NKT cell activation, agonist-mediated human blood and liver MAIT cell activation was bolstered by the presence of type I IFNs.[76,77]

5. **Cytokine-Dominated NKT Cell Activation—**Under certain conditions, NKT cells are activated by the combined actions of IL-12 and IL-18, independent of a CD1d-restricted agonist.[78–80] This mode of NKT cell activation, referred to as cytokine-dominated NKT cell activation (Fig. 3, right panel), is critical for immunity to cytomegalovirus[80] and likely other viruses.

6. Cytokine-Dominated MAIT Cell Activation

MAIT cells were originally thought not to respond to viral infections.[35,37] Subsequent studies demonstrated that viruses—influenza A, hepatitis, and dengue viruses—activated MAIT cells in a mechanism akin to cytokine-dominated activation of NKT cells, i.e., independent of the MAIT cell TCR. MAIT cell activation resulted in a MAIT1 cell-like activity characterized by IFN-γ release and granzyme B upregulation. MAIT cell activation by viruses depended on IL-18, which synergized with IL-12, IL-15 and type I IFNs.[81,82] This form of activation protected mice from lethal influenza virus challenge.[83]

7. ILCs are Activated Solely by the Cytokine-Dominated Mechanism

ILCs lack antigen-specific B or T cell receptors. Their functions are induced in a cytokine-dominated mechanism. The inductive cytokines—which are quite similar to those that promote the differentiation of conventional $CD4^+$ T cells (Fig. 2)—are produced by the local stromal cells and/or APCs, at the site of innate immune response to an injurious stimulus (reviewed in refs. [6,84]).

Thus, ILELs have evolved common and distinct mechanisms to sense perturbations in *milieu intérieur* induced by microbial infections or derived products.

B. Self NKT Cell Agonist/s—One or Many and How are they Made?

Mouse and human NKT cells respond to several self-lipids, including the GSLs as well as the GPLs phosphatidylethanolamine, lysophosphatidylcholine, phosphatidylinositol, and plasmalogen (Table 1, and references therein). How cells biosynthesize GPLs is known, but how mouse and human APCs synthesize αGalCer or iGb3 is not. Current evidence suggests that humans lack the gene encoding iGb3 synthase (the α−3-galactosyltransferase-2), which adds the reducing galactosyl residue onto lactosylceramide to generate iGb3.[85] Whilst mice carry the iGb3 synthase gene, it is expressed only in the dorsal root ganglion. Hence, iGb3 synthase deficiency does not have an impact on NKT cell development or function.[86] These findings are further confounded by conflicting results from different groups as to the presence of iGb3 in mouse cells.[85,87,88] An alternative route of iGb3 biosynthesis involving human A and mouse *cis*-AB enzyme has been suggested (schematized in Fig. 4A; see ref. [89]) but remains unconfirmed.

Another unsolved question pertains to the presence of αGalCer in mammalian cells even though αGalCer-like reactivity has been reported.[56] Whilst β-glucosylceramide synthase (GCS)-deficient thymocytes—which are unable to biosynthesize βGclCer and higher order GSLs—do not promote NKT cell development,[57] β-galactosylceramide synthase (CGT1)-deficient thymocytes do.[61] These results suggested that a βGlcCer-derived GSL is a selecting ligand and implicated iGb3 as a potential ligand.[57]

β-glucosylceramide synthase-deficient cells do not activate NKT cell hybridomas.[56,57,61] Hence, βGlcCer itself or a βGlcCer-derived GSL may be a self NKT cell agonist, which is supported by other evidence.[90,91] One study found that αGalCer or an αGalCer-like compound could be a self NKT cell agonist, but a biosynthetic path to its generation is not established.[56] One possible route to the biosynthesis of αGalCer and αGlcCer might respectively be CGT1 and CGS themselves. These enzymes may have an α-linkage retention property whereby the glycosytransferases use α-linked uridyldiphosphate-charged sugar donors to form β-linked monohexosylceramides by catalyzing α to β mutarotation prior to the condensation reaction. Nonetheless, the potential presence of αGlcCer/αGalCer in the absence of α-hexosylceramide synthase genes within mouse and human genomes poses a quandary.[56,91] As a resolution, it is now recognized that the hexosylceramide synthases can retain the α-linkage of the charged sugar donor to generate α-linked monohexosylceramides (schematized in Fig. 4B). This notion is supported by biochemical evidence.[92–95] Hence, in all likelihood, mammalian GCS and CGT have α-anomer retaining activity.

Cellular lipid content is stringently regulated. Hence, we hypothesized that the ER stress–induced unfolded protein response, which induces *de novo* lipid biosynthesis,[96–99] may activate NKT cells.[100,101] Recent findings have lent support to this hypothesis.[102] Therefore, bacterial and viral infections, either by DC activation through TLR ligation or by ER stress induced by over-expression of virus-derived membrane glycoproteins, have the potential to alter cellular lipid content, both in variety and in concentration. Such changes in cellular

self-lipid content can alter the quality and quantity of the ligands presented by CD1d.[103] Because the NKT cell TCR exhibits co-operative ligand binding,[104] it may recognize subtle qualitative and quantitative changes reflected in CD1d-associated lipid content. We hypothesize that sensitive antigen recognition at very early stages of infection, which may be initiated by very few infectious particles, is key to NKT cell function *in vivo*.

Thus, a model emerges in which CD1d detects alterations in cellular lipid content by virtue of its inherent affinity for such ligands. The presentation of self or non-self α-anomeric glycolipids on CD1d by DCs activates NKT cells. *In vivo* activation of NKT cells induces the prompt secretion of pro- and anti-inflammatory cytokines and chemokines, with the potential to jump start the immune system (Fig. 5). In doing so, the immune system is alerted by the entry of only a few intruders as occurs in natural infections.

C. Steering Innate and Adaptive Immune Responses by Activated NKT Cells

NKT cells form immune synapse upon recognition of CD1d-bound lipid agonists displayed on APCs or planar membranes. The dynamics of the NKT cell TCR/ligand interactions determine the functional outcome.[105] Positive co-operative interaction of NKT TCR with CD1d-lipid agonistic complexes enables NKT cells to sense and respond to subtle changes in cellular lipids.[106] Upon activation, NKT cells rapidly polarize IFN-γ and lytic granules to the immune synapse to transmit an effector response.[105,107,108] The transmission of effector molecules controls downstream innate and adaptive immune responses as described below.

Like the cells of the innate immune system (e.g., neutrophils, Mϕ, DCs, and NK cells), NKT cells respond within the first few hours upon agonist recognition *in vivo* and secrete inflammatory cytokines and chemokines (Fig. 5, left half). To accomplish this feat, NKT cells have epigenetically modified the *Il4* and *Ifng* genes so as to constitutively express both transcripts.[109–113] The nature of the activating NKT cell agonist determines the specific cytokine response (see Table 1). For example, the synthetic agonist αGalCer, within 30–90 minutes, elicits a wide variety of cytokines (Fig. 5, left half), while αGalCer variants containing different lipid chain length or unsaturation typically induce an IL-4 cytokine response.[114,115] In contrast, αGalCer variants modified at distinct linkages induce an IFN-γ response (Table 1 and references therein). Thus, lipid agonists can be harnessed to direct immune responses that induce the desired therapeutic cytokine responses. This feature of αGalCer variants is further accentuated by the ability of activated NKT cells to transactivate cells of the innate and adaptive immune systems as narrated below (see Fig. 5).

DCs, especially CD8α$^+$ DCs, which are major producers of IL-12,[116] are critical for glycolipid agonist presentation, and subsequent NKT cell activation.[117–123] In turn, activated NKT cells further stimulate and induce rapid maturity of the interacting DCs. The result is an upregulation of costimulatory molecules CD40, CD80, CD86, and several receptors necessary for antigen processing and presentation, such as DEC205 and MHC class II molecules, as well as an inflammatory cytokine (TNF-α and IL-12) response.[23,124–127] Activated NKT cells also produce IFN-γ, which coupled with CD154 (CD40 ligand on NKT cells) and CD40 (on DCs) mediate reciprocal NKT-DC interactions.[128,129] This NKT-DC dialogue directs various downstream immune responses, viz.: (1) IL-12 and IL-18 derived from NKT–DC interaction induce IFN-γ production by NK cells[126]; (2) NKT–DC

crosstalk can induce IL-4, IL-6, IL-13, and IL-21 secretion, which cumulatively enhance B cell responses to protein antigens[130–137]; (3) NKT cell–DC interaction also sanctions DCs for antigen cross-presentation to $CD8^+$ T cells,[138–140] as well as the activation and differentiation of CD4 and CD8 T cells.[124,139–141] Thus, the initial and sustained NKT cell–DC interactions amplify and steer downstream innate and adaptive immune responses.

Identification of the transactivation function of NKT cells through cytokine production and contact-dependent mechanisms (Fig. 5)[8,142] led to the idea that these cells act as "cellular adjuvants."[8,143] NKT cell-DC crosstalk was recently shown to instruct inflammasome-independent IL-1β release during bacterial infection, especially by those pathogens that have devised ways to hide from the inflammasome pathway. Infected cells alter cellular lipid content, and NKT cells recognize such alterations when presented by CD1d. NKT cells, so activated, rapidly translocate the pre-existing intracellular pool of FasL to their cell surface, and transactivate Fas on APCs to induce the Fas-associated effector enzyme Caspase-8. Activated Caspase-8 cleave pro-IL-1β converting it to bioactive IL-1β, releasing it from infected APCs in a canonical Caspase-1 and non-canonical Caspase-11 inflammasome-independent manner.[144] Caspase-8 also cleaves pore forming protein gasdermin D (GSDMD), resulting in GSDMD pore formation, K^+ efflux and finally activation of the NLRP3-Caspase-1 inflammasome to further amplify IL-1β secretion.[143] This two-cell back-up model for IL-1β release stymes pathogens leaving them no place to hide.[145] Together, these findings provide a physiological context to NKT cell-APC crosstalk, bridging the topological barrier between the site of infection, sensory innate response, and T cell activation. Further, activated NKT cells provide context to downstream innate and adaptive effector responses.

D. Division of Labor by Innate-Like Effector Lymphocyte Subsets

Innate-like effector lymphocyte activation results in rapid secretion of pro-inflammatory and regulatory cytokines and chemokines. Congruent with their ability to transactivate a range of innate and adaptive immune cells (see Fig. 5), ILELs control downstream immune responses. NKT cells are heterogeneous, consisting of at least three subsets—NKT1, NKT2, and NKT17[146]—as well as at least two induced subsets, NKT10 and NKTfh.[133,134,136,147–150] Analagous subsets are also well characterized in ILCs and MAIT cells (although a MAIT2 cell subset remains as yet unidentified), and are emerging in γδT cells as well.[5,7,151] Akin to conventional $CD4^+$ T cell subsets, ILEL subsets are defined by subset-specific transcription factors and archetypal cytokine responses that, respectively, mediate type 1, type 2, and type 3 immunity (see Figs. 2 and 5). Each subset is represented in different proportions in different mouse strains, and varies within each strain at barrier tissues (Fig. 6).[146–148,151–154]

TYPE 1 innate-like effector lymphocytes include NK cells, ILC1, γδT1 cells, NKT1 cells, and MAIT1 cells (Figs. 2 and 6). NKT1 cells activation results in a Th1-like cytokine response. Most NKT cells are of the NKT1 subset in C57BL/6 mouse thymus, spleen, and liver. NKT1 cell differentiation is dependent on T-bet (*Tbx21*) and IL-15, and to a limited extent, GATA3.[146,152,153,155–157] Unlike in DP thymocytes, NKT cell lineage-specific depletion of HDAC3 selectively impairs NKT1 cell development as a result of

diminished autophagy[158–160]—a cytoplasmic recycling process essential to both T and NKT cell development—as well as reduced expression of nutrient receptors GLUT1, CD71, and CD98.[161] NKT1 cells mediate the anti-tumor effect of αGalCer,[162] potentially through secreted IFN-γ and TNF-α.

TYPE 2 innate like effector lymphocytes include ILC2, γδT2 cells, and NKT2 cells (Figs. 2 and 6). MAIT2 cells have not been identified.[5,151,163] γδT2 cells are enriched in Tec kinase deficient mice.[164] NKT2 cells express the CD4 co-receptor, are enriched in mouse lungs and intestine, and their activation results in a type 2 cytokine and chemokine response (Fig. 6). This type 2 response may underlie airway hyperresponsiveness,[152,153,165–168] a prominent feature of asthma, as a result of macrophage, eosinophil, neutrophil, and lymphocyte recruitment into the lungs and consequent tissue damage.[165] Coincidently, NKT2 cells are overrepresented in BALB/c mice that are sensitive to airway hyperresponsiveness.[153]

TYPE 3 innate like effector lymphocytes include ILC3, γδT17 cells, NKT17 cells, and MAIT17 cells (Figs. 2 and 6). NKT17 cells are prominent in the peripheral lymph nodes, skin, and lungs, but scant in the liver and spleen (Fig. 6).[169–171] NKT17 cells need IL-7 but not IL-15 for survival,[152,172] and their development requires mTORC2 signaling and the transcription factors Runx1 and NKAP.[173–177] Indeed, *Runx1* depletion in NKT cells results in decreased IL-7Rα, BATF, and c-Maf expression, and increased Lef and Bcl11b expression.[175] The role of NKAP in NKT17 cell development is not understood; it appears not to require mTOR, IL-7, or TGF-β signaling.[173] NKT17 cells constitutively express RORγt,[169] promote airway neutrophilia upon challenge with synthetic glycolipids or LPS, and rapidly secrete IL-17A in response to some bacterial infections.[28,169,178] They may mediate ozone-induced airway hypersensitivity,[179] experimental autoimmune encephalomyelitis,[178] and acute hepatitis in mice.[180]

MOUSE NKT10 cells, the PLZF-independent subset,[147] are found in low frequency in naive mice and in human peripheral blood. Upon re-activation, NKT10 cells that formerly responded to αGalCer *in vivo* secrete IL-10,[148] which is thought to sustain immune-privileged sites. This NKT cell subset may also regulate T_{REG} cell activities in adipose tissues.[147]

MOUSE NKT follicular helper (NKT_{FH}) cells can provide cognate (when B cells present lipid antigens) or non-cognate (when B cells present protein antigens) help to B cells and regulate antibody responses.[133,134,136,149,150] *In vivo* αGalCer induces a subset of NKT cells to take on a T follicular helper T (TFH) cell-like phenotype, forming NKT_{FH} cells.[150,181,182] NKT_{FH} cells express CXCR5, PD1, BTLA, ICOS, and Bcl6. Their development depends on the same factors that induce T_{FH} development.[181] NKT_{FH} cells–produced IL-21 rapidly drives germinal center formation, yielding appreciable levels of antigen-specific IgG antibodies.[135,181,182] Nonetheless, NKT_{FH} cell–induced antibody responses are ephemeral and inferior to T_{FH} cell-driven antibody responses.[135,181,182] NKT_{FH} cells may control antibody responses against *Borrelia hermsii*, *Streptococcus pneumoniae*, and *Plasmodium falciparum*—all of which are human pathogens.[135,181,182] NKT_{FH} and T_{FH} cells can synergize to promote robust antigen-specific antibody responses, thus highlighting the utility of αGalCer as a vaccine adjuvant.[150]

HUMAN NKT cell responses are as diverse in humans as in mice,[183] yet subsets similar to those in mice were only recently described. Previous reports have demonstrated that human $CD4^+$ and double-negative (DN) NKT cell subsets are functionally different. Activated human $CD4^+$ NKT cells, in a pathologic role, accumulate in the lungs of chronic asthmatic patients; these NKT cells produce IL-4 and IL-13.[184] Hence, human $CD4^+$ NKT cells mirror the mouse NKT2 cell subset. Moreover, activated DN NKT cells produce IFN-γ and TNF-α and, thereby, mirror mouse NKT1 cells. Also, in the presence of inflammatory signals, both $CD4^+$ and DN human NKT cell subsets induce perforin production. DN NKT cells also upregulate NKG2D expression, which combined with perforin may mediate cytotoxicity against infected cells and cancer cells.[185,186] These human NKT cell functions parallel those of mouse NKT1 cells. Moreover, the presence of an NKT17-like subset is indicated by the finding that activated human NKT cells also secrete IL-17.[183] Consistent with these findings, human liver perfusates contain both NKT, MAIT, and γδT cell subsets. Whilst NKT17, MAIT17, and γδT1 cells dominate the liver, proportionately low frequencies of NKT1, MAIT1, and γδT17 also are present.[187] These subsets produce characteristic cytokines as do the mouse counterparts: the type 3 subsets produce IL-17, and the type I subsets, IFN-γ.[187]

Overall, ILELs divide labor among three distinct subsets. Specifically, global and single cell transcriptome analyses demonstrated that thymic NKT1, NKT2, and NKT17 cells represented definite subsets.[187–190] While not formalized, human NKT cells potentially mirror mouse NKT cell subsets,[187] hence requiring further enquiry. Differentiation of NKT cell subsets is influenced by tissue environment. Hence, NKT17 differentiation requires mammalian target of rapamycin (mTOR) complex-2[177] and is inhibited by Tet enzymes that modify DNA 5-methylcytosine by controlling the expression of the Tbet and ThPOK transcription factors.[191] A separate study using mice with monoclonal NKT cell populations showed that tissue homing pattern, but not NKT TCR avidity, dictated NKT cell subset differentiation.[171] By contrast, other studies have found a significant role for TCR avidity in subset development.[192–194]

III. HOW NKT CELLS GOT THE WAY THEY ARE

A. Evolutionary Origins

> "…*the struggle against diseases, and especially infectious diseases, has been a very important evolutionary agent and that some of its results have been unlike those of the struggle for life*…" ([195] within collected papers in genetics by J.B.S. Haldane[196]). Nonetheless, "*There is a natural and irrepressible tendency in the human mind to penetrate the mystery of the beginning of things…. But it is plainly denied to finite understandings to ascend to the very beginning, and to comprehend the nature of the operation of the First Cause of anything…. The ablest endeavours here to penetrate the beginning of things do but carry us, when most successful, a few steps nearer that beginning, and then leave us on the verge of a boundless ocean of the unknown truth, dividing the secondary or subordinate phenomena in the chain of causation from the great First Cause.*"[197]

Sir Owen, a vocal critic of Darwin's evolutionary theory by natural selection,[198] may have been a bit too short-sighted with the above critique of the *Origin*. The exciting new biology of the 21st century knows no bounds. The essay below provides a glimpse on how one could "penetrate the mystery of the beginning of things" eons past!

Recent advances in whole genome sequencing (WGS) have ushered in comparative vertebrate genomics unveiling molecular signatures of selection upon genes that control various biologic functions, including immune responses. Pathobionts can apply inordinate selection pressure and significantly impact the evolution of immune response genes and cells. As early-life symbionts can impact health, microbial ecology may also control the evolution of immune response genes and cells.

The NKT cell and MAIT cell TCR engage their ligands with conserved germline-encoded residues in the complementarity-determining regions.[199] Hence, phylogenetic analyses of the genes and gene segments that code for these molecules can inform their origins and whether selective pressures may have maintained them over evolutionary scales of time. Such analyses indicate that the *MR1* and *Cd1d* genes, as well as the *TRAV1* and *TRAJ33*, and *TRAV10* and *TRAJ18* gene segments that code for MAIT cell and NKT cell TCRs, respectively, evolved in a common ancestor/s of the therian mammals, before the metatherian (viviparous marsupial mammals) and eutherian (viviparous placental mammals) split but after the monotreme (oviparous mammals) therian split some 170 million years ago. Further, *MR1*, and *TRAV1* and *TRAJ33* genes may have co-evolved with each other. Curiously, however, not all eutherian (placental) mammals carry *MR1* and *Cd1d* genes, as well as the *TRAV1* and *TRAJ33*, and *TRAV10* and *TRAJ18* gene segments. Whilst the *MR1* gene has evolved slowly, in comparison, *Cd1* genes have evolved faster and diversified since their appearance in eutherian mammals. These findings are suggestive of selection pressure, presumably by riboflavin-producing microbial pathogens, in the maintenance of *MR1*, and *TRAV1* and *TRAJ33* genes.[200]

The above study did not find evidence for NKT cells or MAIT cells in other vertebrate animals.[200] Nonetheless, there are reports of innate-like T cells in *Xenopus laevis* that look and function like NKT and MAIT cells.[201,202] Notably, the search for *MR1*, and *TRAV1* and *TRAJ33* genes led to the discovery of co-evolving *MR1*-like, and *TRAV1*- and *TRAJ33*-like genes, *TRAV41* and *TRAJ38* genes in rabbits.[200] Rabbits along with other lagomorphs, carnivores, and armadillos lack the *MR1* gene. Thus, there are other potential ways to generate MAIT cells, lending credence to the idea that the *Xenopus* innate-like T cells may be another such example. This conjecture awaits formal evidence.

Cd1 and *MR1* genes are relatively young when compared to the classical antigen-presenting *MHC* genes, *Cd1* being older than *MR1*, the latter evolving around the same time as *Cd1d*. *Cd1* was an amniote innovation, evolved in Mesozoic reptiles and sustained in the extant reptiles, such as the anapsid green anole lizard *Anolis carolinensis* and synapsid Siamese crocodile *Crocodylus siamensis* and Chinese alligator *Alligator sinensis*.[203] *Cd1* genes diversified in mammals, wherein evolved *Cd1d*.[200] The reptilian *Cd1* gene lacks orthology to avian or mammalian *Cd1* genes,[203] indicating that *Cd1* genes may have risen several times in amniote evolution. Alternatively, *Cd1* genes may have rapidly evolved and

considerably diverged from the reptilian form within extinct synapsid and mammal-like reptiles prior to equilibration within eutherian species. The absence of *Cd1* in oviparous monotremes such as platypuses, and the presence of a *CD1d*-like gene in a few metatherians such as the opossum, supports the alternative view to *Cd1* evolution.

A phylogenetic analysis of TRAV10 (encoding the human Vα24 gene segment) or TRAV11 (encoding the mouse Vα14 gene segment) and TRAJ18 (encoding the Jα18 gene segment) indicated that gene elements related to TRAV10/11 and TRAJ18 are found solely in placental mammals.[200] This discovery indicates that NKT cells are a eutherian contraption. As the host gut microbiota influences NKT cell terminal functional differentiation,[27,33] and NKT cells can control gut microbial ecology,[204] we predict that placental development, sudden perinatal exposure to maternal and environmental microbiota, and lactation may have contributed to the evolution of CD1d-restricted NKT cells. The same might be the case for MAIT cells[200] and other ILELs, as they all recognize and/or respond to microbes, both symbiotic and pathogenic.

γδT cells are found in jawed (gnathan) vertebrates including cartilaginous (Chondrichthyes: rays, sharks, and skates) and bony (Osteichthyes: salmon, tuna, and zebra fish) fishes.[51] γδT cells in these species use V(D)J recombined TCRs. Similarly, γδT-like cells are found in jawless (agnathan, e.g., lampreys and hagfish) vertebrates that use leucine-rich-repeat–based variable lymphocyte receptors, which also randomly rearrange in a Rag1/Rag2-independent mechanism to generate antigen-specific receptors.[205,206] At the present time, it is not known whether these γδT cells are innate-like γδT or γδNKT cells.

Another means to penetrate the evolutionary beginnings of ILELs is the use of a comparative transcriptomics approach as previously described. This approach was applied to uncover the origins of NK cells—the natural cytotoxic ILCs (Fig. 2)—and T helper-like ILCs. Lineage specific inducers (soluble mediators), surface markers, and transcription factors trace back some 500 Mya, suggesting the origins of NK cells and ILC2s may have been conceived at the dawn of vertebrate evolution. ILC1 and ILC3 appeared more recently, but a clear point in time for their origins needs further study.[207] Single cell transcriptomics[190] shall illuminate their origins and firm those established. Further, such approaches could unveil any lymphoid lineage/s that may have preceded the likes of NKT and MAIT cells. Similarly, single cell transcriptomics can reveal the origins of γδT and γδT-like ILELs and whether the *Xenopus* NKT-like and MAIT-like cells are bona fide ILELs even though they use distinct restriction elements and TCRs.

B. Developmental Origins: Genomic Control of NKT Cell Ontogeny

> "*The child is the father of a man*." From "My Heart Leaps Up When I See a Rainbow in the Sky," a poem by William Wordsworth

NKT cell, MAIT cell, and γδT cell development occurs in the thymus. As with all developmental processes, ILEL ontogeny depends on modular genome regulatory network/s composed of key apex transcription factors and downstream effectors. Thus, to become a T lineage ILEL versus an ILC rests upon the actions of a few apex transcription factors: NOTCH-TCF1-E2A and the E2A regulator Id2 (inhibitor of DNA-binding 2). NOTCH1

effectors TCF1 (encoded by *Tcf7*) and E2A (encoded by *Tcf3*) control *Tcf12* (encoding HEB); HEB in turn regulates the expression and rearrangement of *TCR* gene loci, including *TRA11*02* to *TRAJ18* gene rearrangement. The expression of Id2 down regulates *Tcf3* and, thereby, NOTCH1-TCF1 T cell apex transcription factors, begetting the ILC lineage.[208,209]

Commitment to the γδT innate-like cell lineage occurs prior to γδT innate-like lineage commitment. A key nuclear event that begets the ILEL-lineage and associated innate-like functions involves the activation of the zinc finger BTB domain-containing-16 (*Zbtb16*) gene that codes for promyelocytic leukemia zinc finger (PLZF). PLZF-mediated genomic control differentiates the unique NKT, MAIT, and γδT cell functions from those of other T lymphocytes. This section covers what we know of NKT cell development, much of which is similar to MAIT cell development, and perhaps γδT cell ontogeny as well.[5] Developmental plans of other ILELs are reviewed elsewhere.[5,6,51,209–211]

Genetically modified mice whose thymocytes fail to develop beyond the (DN)2/DN3 stage also lack NKT cells (Fig. 7).[54,212–216] Further, NKT cells fail to develop in mice with mutations in *Myb* (coding for c-Myb), *Rorc* (coding for RORγt), and *Tcf12*.[217–220] As *TRAV11*02* and *TRAJ18* rearrangement occurs at a late DP stage,[217,219] and NKT cells develop in *Jα18*-deficient mice that received highly purified tetramer-negative, DP-high thymocytes,[221] commitment to the NKT cell lineage occurs at the DP stage.[222] *TRAV11*02* and *TRAJ18* rearrangement, however, can occur within late DN thymocytes and the resulting precursors can differentiate into NKT1 cells.[223]

Commitment to the innate-like T cells occurs shortly after positive selection by thymic self agonists, and proceeds through defined stages and culminate in branched differentiation to three major subsets shown in Fig. 7. The ligation of the invariant NKT cell TCRα (iTCRa) by its cognate agonist on DP thymocytes induces *Nr4a1* expression encoding Nur77, which lasts for about two days.[224] Shutting down *Nr4a1* expression is critical because Nur77 can induce cell death to anergy in developing NKT cells (discussed below; ref. [194]). The mechanism of *Nr4a1* shutdown, albeit unknown, perhaps results from dissociation from the agonist or by signal/s relayed by the SLAM-SAP-Fyn module, which is known to temper iTCRa signal intensity.[193,224] Coincident with Nur77 depletion is the upregulation of *Zbtb16* expression, encoding PLZF.[224] It is not known whether positive selection results in two stage 0 (st0) subsets—one with low and the other with high Nur77, the latter destined to deletion by apoptosis. St0 cells with low Nur77 may then proceed to st1 as PLZF levels increase. Alternatively, SLAM-SAP-Fyn activation in st0 cells may bolster PLZF expression and repress *Nr4a1* locus to prevent deletion and to permit onward development. Whichever be the mechanism, st1 cells committed to the lineage undergo proliferative burst and expand and upregulate CD44,[187,190,224] becoming permissive to differentiation. NKT1 cells differentiate under the influence of IL-15 (and potentially type I IFN[190]) *trans*-presented by medullary stromal cells interacting with NKT cell precursors.[155–157,225,226] Those that do not receive IL-15 and/or type I IFN signal/s or are not permissive to these signals beget st2 cells, which are precursors to NKT17 and NKT2 cell subsets. Whilst IL-7 receptor signaling programs NKT17 cell differentiation, signals that promote NKT2 cell differentiation is unknown. Alternatively, NKT2 cell subset could be default path that needs no specific signal/s. This NKT cell ontogenetic and differentiation pathway, extracted from

recent reports,[187,190,227] resembles the Waddington epigenetic landscape[228,229] of binary cell fate decisions made by an immediate precursor until end-stage differentiation occurs. Furthermore, recent reports suggest that MAIT cell and γδT cell development and subset differentiation follow the same general principle as those described above.[187,190]

Mechanism/s of effector subset differentiation is incompletely understood. Evidence support a iTCR-agonist affinity-based model coupled with cytokine signaling,[190,192,193] tissue homing site-specific,[171] and sole cytokine-based differentiation.[224] The induction of CD69 at the late stage of NKT cell differentiation[190,224] has suggested a iTCR-agonist affinity-based differentiation model. Despite CD69 induction, which results from TCR activation, no induction of Nur77 —a proxy for TCR activation—was observed. IFN-I signaling can induce CD69, and IFN-I-regulated genes are induced in NKT1 cells.[190,230] These findings support a cytokine-based differentiation model. It is possible that a low affinity iTCR ligand, which may not induce Nur77,[231] sufficiently tickles the NKT cell TCR to make NKT cell precursors responsive to cytokine signaling. These competing models need further resolution.

Unlike conventional $CD4^+$ and $CD8^+$ T cells, which depend on thymic epithelial cells for positive and negative selection, $NK1.1^+$ T cells rely on DP thymocytes for positive selection.[232] NKT cell TCRs expressed by precursors interact with self lipid-bound CD1d on DP thymocytes[233–237] and by homotypic signaling lymphocytic activation molecule (SLAM)-SLAM receptors on both cell types.[238–240] These molecular interactions result in the activation of protein kinase Cθ-NF-κB and NFAT-Egr2 signaling modules, culminating in the induction of specific transcriptional programs crucial for NKT cell maturation (Fig. 7).[238,239,241–246] PLZF-mediated transcriptional programs bestow upon NKT cells its unique functions through a differentiation process that results in NKT1, NKT2, and NKT17 cell subsets and the acquisition of cytokine secretion function.[190,242,247,248] Whilst the NKT cell subsets are defined by the same subset-specific transcription factors expressed by the corresponding T helper cell subsets, NKT cells differ from T helper cells in the ability to quickly secrete cytokine/s in response to activation (Fig. 2).[147,148,152–154,188]

Gene regulatory networks (GRNs) control lineage-specific gene expression and unveil the developmental and evolutionary origins of cell lineages.[249] PLZF is a lineage-specific master transcription factor, functioning as a critical node in the GRN that controls innate-like effector differentiation in developing NKT cells.[247,248,250] *Zbtb16* induction is in part regulated by acetylated Egr2[251] induced downstream of NKT cell TCR signaling.[244] A recent study revealed that the histone acetylase GCN (general control non-derepressible) 5 acetylates a critical lysine residue in Egr2, and DP thymocyte-specific depletion of GCN5 abrogated the progression of NKT cell development from st0 to st1 in a cell intrinsic manner. This st0 to st1 developmental block was secondary to the transcriptional downregulation of *Zbtb16* and other essential NKT cell development genes such as *Runx1*, *Tbx21*, and *Il2rb*.[251] GCN5 itself is an acetylated protein. Whether its function during NKT cell development depends on acetylation is currently unknown. In some models, the function of GCN5 depends on its deacetylation.[252] Should GCN5 function in NKT cells depend on deacetylation, whether and which sirtuins (silent mating type information regulation 2 homologs 1–7; ref [252]) play this role in NKT cells remains to be established.

While the mouse NKT cell iTCRa can pair with virtually all available TCR β-chains, the peripheral NKT cell repertoire consists of iTCRa paired with a restricted set of β-chains, viz., Vβ8, Vβ7, and Vβ2.[253–256] It is generally thought that the semi-invariant NKT cell TCR repertoire is built solely by positive selection.[257] This assumption is at odds with high affinity interactions between the NKT cell TCR and its cognate self-agonist, which results in self-reactive T cells. Hence, indirect evidence has implied a role for negative selection in sculpting the semi-invariant NKT cell TCR repertoire.[258–262] Evidence in favor of both models has been discussed elsewhere.[142]

Nr4a1-encoded Nur77,[263] a member of the orphan nuclear receptor transcription factor family,[264,265] is expressed in both precursor and peripheral agonist-activated NKT cells.[192,243,263] Nur77 expression is induced downstream of NKT cell TCR activation.[192,243,263] Nur77 aids in negative selection of conventional T cells by converting the pro-survival factor Bcl-2 to a pro-apoptotic agent.[264,266–269] Thus, a recent study found that Nur77 overexpression via a thymocyte-specific *Nra4* transgene arrested NKT cell development at an early precursor stage shortly after positive selection, and induced negative selection and self-tolerance in thymic NKT cells, making these cells hyporesponsive to agonistic stimulation in the periphery.[194] Moreover, homotypic SLAM-SLAM interactions between iNKT cell precursors and DP thymocytes are critical for the selection and differentiation of this lineage.[232,238,241,270–276] Hence, this interaction is fine-tuned at the level of SLAM family member expression on the two cell types.[277] NKT cells that develop in mice deficient in all six SLAM family receptors recapitulated the Nur77 overexpression phenotype.[193] These findings together lend to a model in which strong and persistent NKT cell TCR signaling after positive selection induces high Nur77 expression. Because Nu77 induces apoptosis, precursors can become dead end cells as seen in NKT cells that overexpress Nur77 (Fig. 7; alternate paths at st0). Instead, the induction of SLAM family receptor signaling overrides Nur77-induced apoptosis and facilitates onward iNKT cell development. Consistent with this model, increased SLAM (SLAMF1) and Ly108 (SLAMF6) expression in st0 NKT cells could not temper Nu77 effects, perhaps because Nur77 overexpression was driven by the *Lck* proximal promotor and not the native *Nr4a1* promotor. The next step would be to elucidate how the SLAM-SAP-Fyn signaling module controls *Nr4a1* expression and function. Thus, both positive and negative selection events sculpt NKT cell TCR repertoire.

IV. END NOTES: A “LIMBIC IMMUNE SYSTEM” EMERGING IN PLAIN SIGHT

PLZF directs the distinct behaviors of a group of ILELs including γδT cells, NKT cells, MAIT cells, and ILCs.[278–280] Also, PLZF is required for NK cell function, but not NK cell development.[281] On the other hand, symbionts (gut and potentially other barriers such as skin and lungs) determine the development (MAIT cells, and potentially γδT cells) and functional differentiation (NKT cells, MAIT cells, and ILCs) of ILELs. We propose that these immune cells, all of lymphoid origin, be classified as the “limbic immune system” as they function at the edge (*limbus* in Latin) of the innate and adaptive immune systems. Remarkably, γδT, NK, and NKT cells localize to the interfollicular region of the lymph

nodes, in between cells of that make up the innate and adaptive immune systems.[282] Whilst not controlled by PLZF or the microbiota, by virtue of their physiologic functions, other tissue-restricted innate-like lymphocytes, such as CD8αα innate-type lymphocytes[283] as well as B1 cells and NK cells,[284] can be included in the limbic immune system. In its simplest form, the limbic immune system is anglicized Latin for the "in-betweeners"[285] and, hence, synonymous with it.

In this proposal for a triune immune system we make no claims or assumption that the limbic immune system is an evolutionary transition between the innate and adaptive immune systems. But, instead, the limbic immune system is a conglomeration of independently acting modules, arising at different times in evolution, in many instances, repurposing loosely common genome regulatory circuits to accomplish a common task: to integrate information relayed by the innate sensory immune system about the local tissue environment and to provide context to downstream effector innate and adaptive immune responses. The multiple modules add robustness and evolvability to this limbic system to keep abreast of the ever-changing environment and the quick-evolving microbial cosmos, especially of those members of an otherwise symbiont community that turn pathobiont without much notice!

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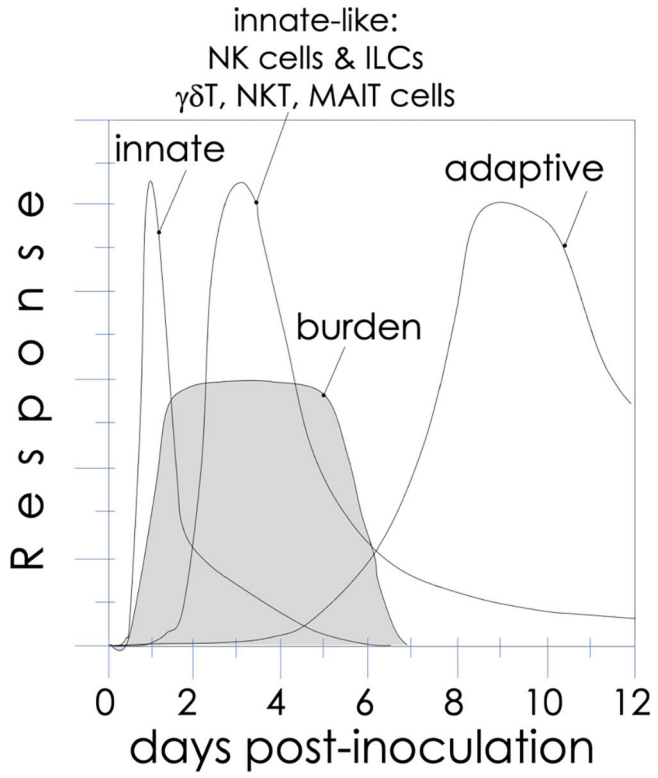


FIG. 1:

The kinetics of an immune response. The first peak signified by “innate” indicates the response of tissue macrophages and immature dendritic cells, which mature over the first few days following microbial (or antigenic) challenge to ferry antigen to local draining lymph nodes. Both also secrete innate cytokines and chemokines to recruit neutrophils. These sensory innate responses are integrated by innate-like effector lymphocytes, whose activities peak after the innate immune response, and relay context to the adaptive immune system T and B cells, impacting the antigenic burden.

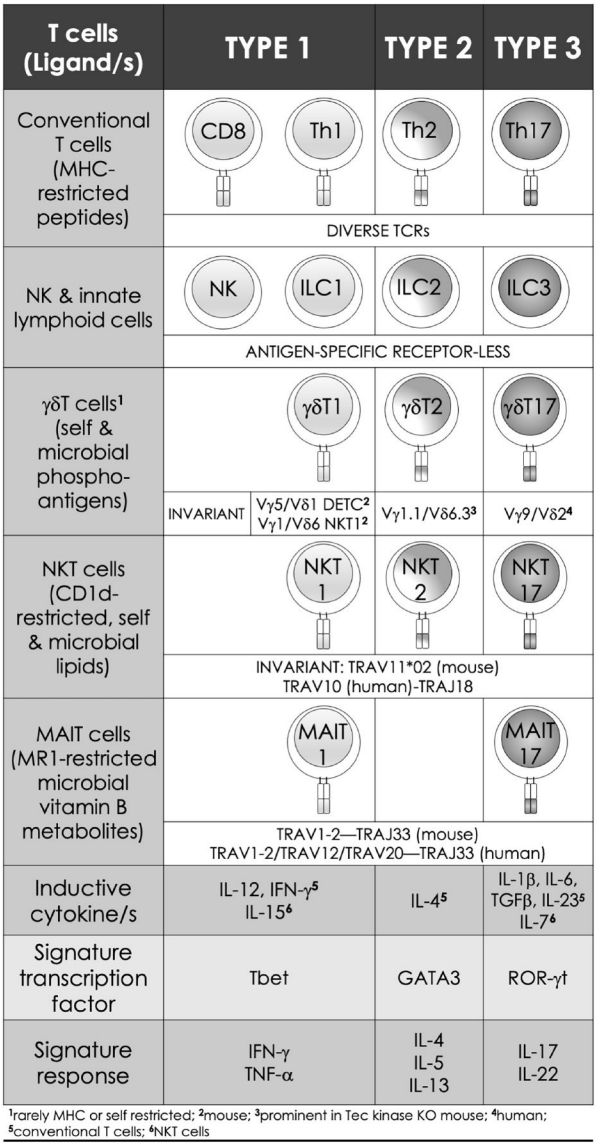


FIG. 2:

Features of innate-like effector lymphocytes mirror those of conventional T lymphocytes. Three types of effector immune responses are recognized: type 1, type 2, and type 3. They are characterized by the absence (ILCs) or presence of antigen-specific receptors on lymphoid lineage cells. Whilst conventional T cells express a diverse TCR (noted in the current IMGT nomenclature) repertoire, those of the innate-like effector lymphocytes express semi-invariant (NKT and MAIT cells) to invariant (γδT cells) TCRs. Type 1 effectors include both innate (NK) and adaptive cytotoxic ($CD8^+$ T) cells and non-cytotoxic T helper (Th) 1 cells, as well as innate-like effector lymphocytes such as ILC1, NKT1, MAIT1, and γδT1 cells. They require IL-12 for induction, which is bolstered by IFN-γ. T-bet and related eomesodermin transcription factors control the differentiation of type 1 effector cells, which are essential for immunity against intracellular pathogens. Type 2 effector cells include Th2, ILC2, NKT2, and γδT cells. These cells are activated by IL-4 and require GATA3 for their effector differentiation. Type 2 effector cells secrete IL-4, IL-5,

and IL-13, which are required for parasite expulsion. Their over activity results in allergic response and hypersensitivities. Type 3 effector cells include Th17, ILC3, NKT17, MAIT17, and γδT17 cells. These effector cells are induced by IL-6, TGF- β, IL-1 β, IL-23, and IL-7 (NKT17). RORγt is the lineage specific transcription factor. Type 3 effector cells secrete IL-17 and IL-22, which are important for immunity to extracellular bacteria and fungi.

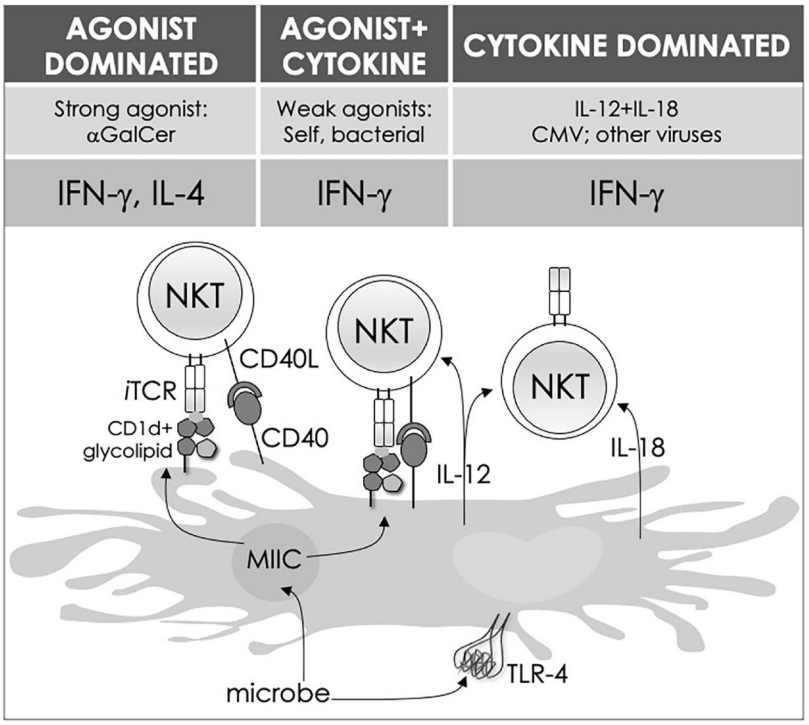


FIG. 3:

Three distinct strategies activate mouse NKT cells. Potent NKT cell agonists—such as αGalCer—directly activate NKT cells without the need for a second signal, in a TCR-signaling dominated fashion (left panel). Alternatively, microbes containing TLR ligands such as LPS activate NKT cells by inducing IL-12 production by DCs, which amplifies weak responses elicited upon recognition of CD1d bound with self-glycolipids by the NKT cell TCR. Several endogenous lipid agonists have been identified and characterized (see Table 1). Some microbes such as *Sphingomonas capsulata*, which are α-Proteobacteria, synthesize α-anomeric glycolipids for their cell walls. These glycolipids, when presented by CD1d, weakly activate NKT cells directly. In the presence of a second signal—generally a pro-inflammatory cytokine such as IL-12—such weak agonists strongly activate NKT cells (middle panel). Intriguingly, NKT cells can be activated solely by cytokines—mainly IL-12 plus IL-18—in a TCR-independent manner (right panel). Similar strategies activate MAIT cells as well. ILCs, owing to the lack of antigen-specific receptors, use cytokine-dependent mechanisms for initiation of an immune response. This diagram rendering the different strategies to NKT cell activation is an adaptation of past reviews[10,101] and is based on works cited in the text.

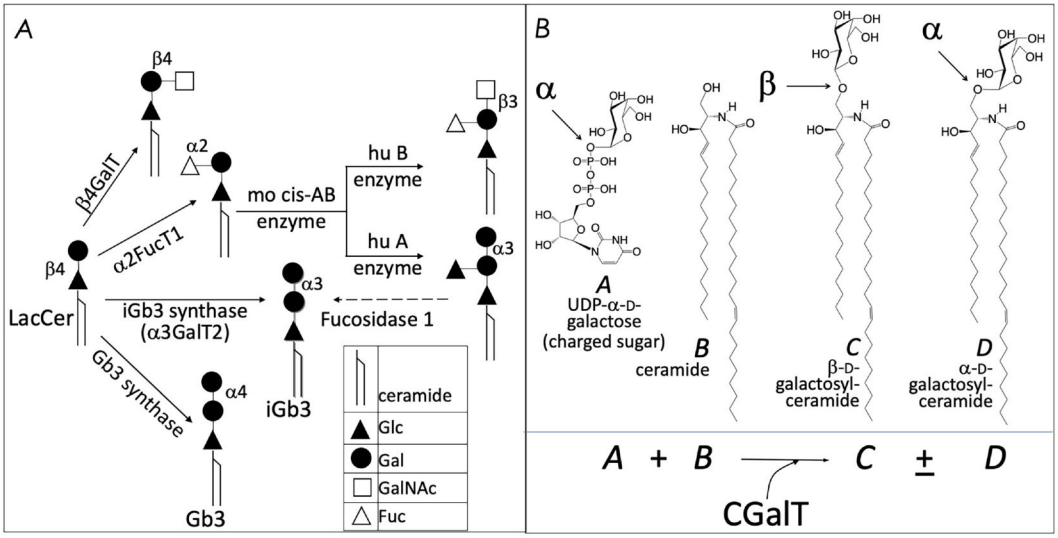


FIG. 4:

Alternate enzymatic strategies for the generation of cellular isoglobotriaosylceramide (A), and α GalCer (B). See text for details.

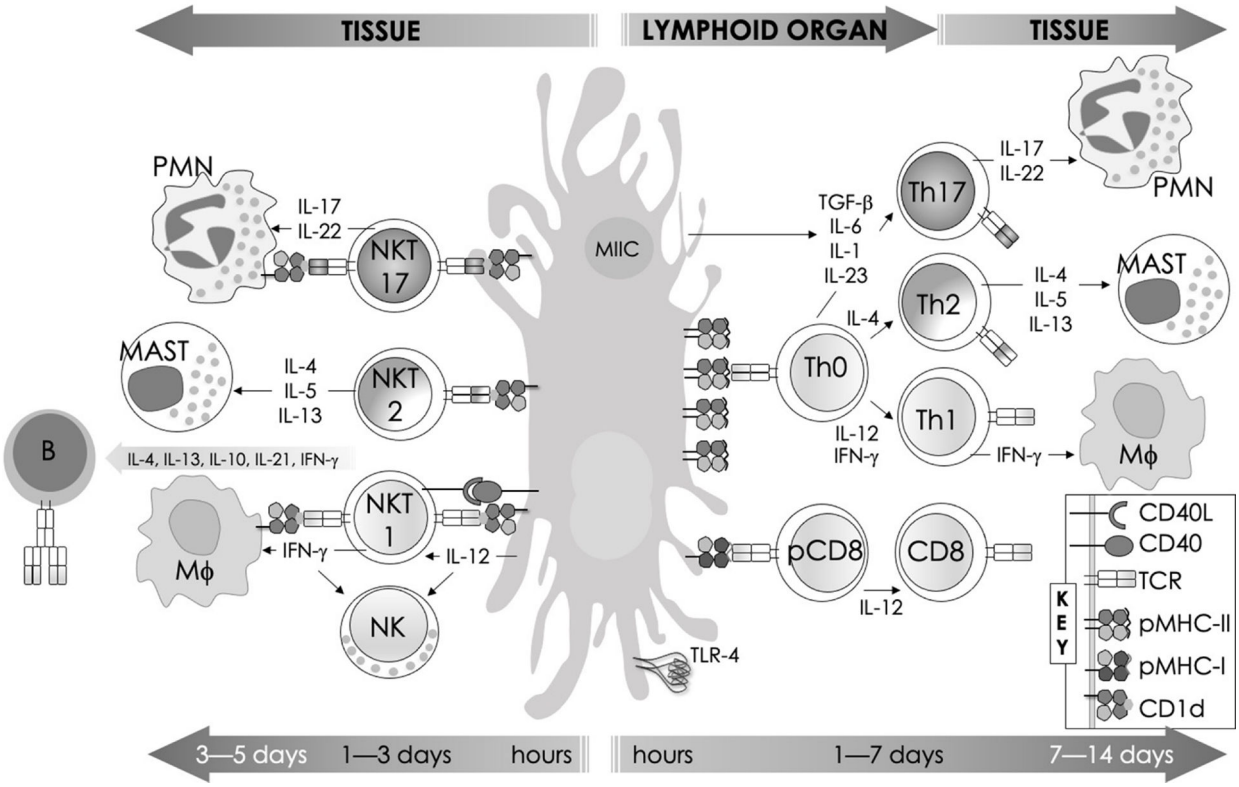
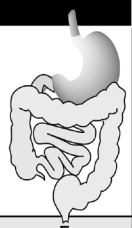
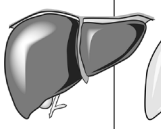
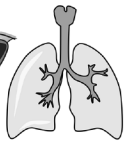
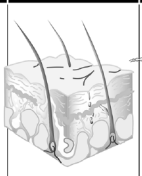
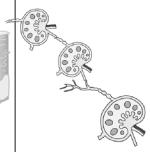


FIG. 5:

Tissue distribution of innate-like effector lymphocytes. See text for details.

	THYMUS	GUT	LIVER	LUNGS		SKIN	LYMPH NODE	SPLEEN
								
ILC 1[1]		5		8		61		
ILC2		31		89		30		
ILC3		64		3.5		9		
NKT1[2: a—d]	15—75	45—80	≥95	20—60	10—60		~5—65	~40—90
NKT2	10—65	3—60	1—3	3—10	50—60		~10—80	~2—55
NKT17	<5—20	1—7	<1—2	25—75	5—25		<1—80	<1—10
MAIT1[3: e,f]		<1	65—>95	70—95	5—30	<10	~30	60—>95
MAIT17		>99	<5—35	5—30	70—95	>90	~70	<5—40

organs, adapted from ChemDraw 20.1; frequencies of the indicated cells in %; blank, not known
[1]ref [6]; [2]approximated based on figure 3B in ref [146] in C57BL/6, BALB/c, & NOD strains, [a]range: intraepithelial lymphocytes, Peyer's patches, & lamina propria lymphocytes of the gut, lung [b]vascular *versus* [c]interstitium, [d]range: mesenteric, inguinal, axillary, & cervical, lymph nodes; [3]approximated from ref [163], [e]colon lamina propria, [f]pooled lymph nodes

FIG. 6:

The effector functions of mouse NKT cells. The interactions between the invariant natural killer (NKT) cell receptor and its cognate antigen, as well as interactions between co-stimulatory molecules CD28 and CD40 and their cognate ligands CD80/86 (B7.1/7.2) and CD40L, respectively, activate NKT cells. Activated NKT cells participate in crosstalk with members of the innate and the adaptive immune systems by deploying cytokine and chemokine messengers. Upon activation *in vivo*, NKT cells rapidly secrete a variety of cytokines and chemokines, which influence the polarization of $CD4^+$ T cells toward T helper (Th)1 or Th2 cells as well as the differentiation of precursor $CD8^+$ T cells to effector lymphocytes, and B cells to antibody-secreting plasma cells. Some of these mediators facilitate the recruitment, activation, and differentiation of macrophages and DCs, which results in the production of IL-12 and possibly other factors. IL-12, in turn, stimulates NK cells to secrete IFN-γ. Thus, activated NKT cells have the potential to enhance as well as temper the immune response. This schematic rendition of NKT cell effector functions is an adaptation of past reviews[8,10,101,296] and is based on works cited in the text.

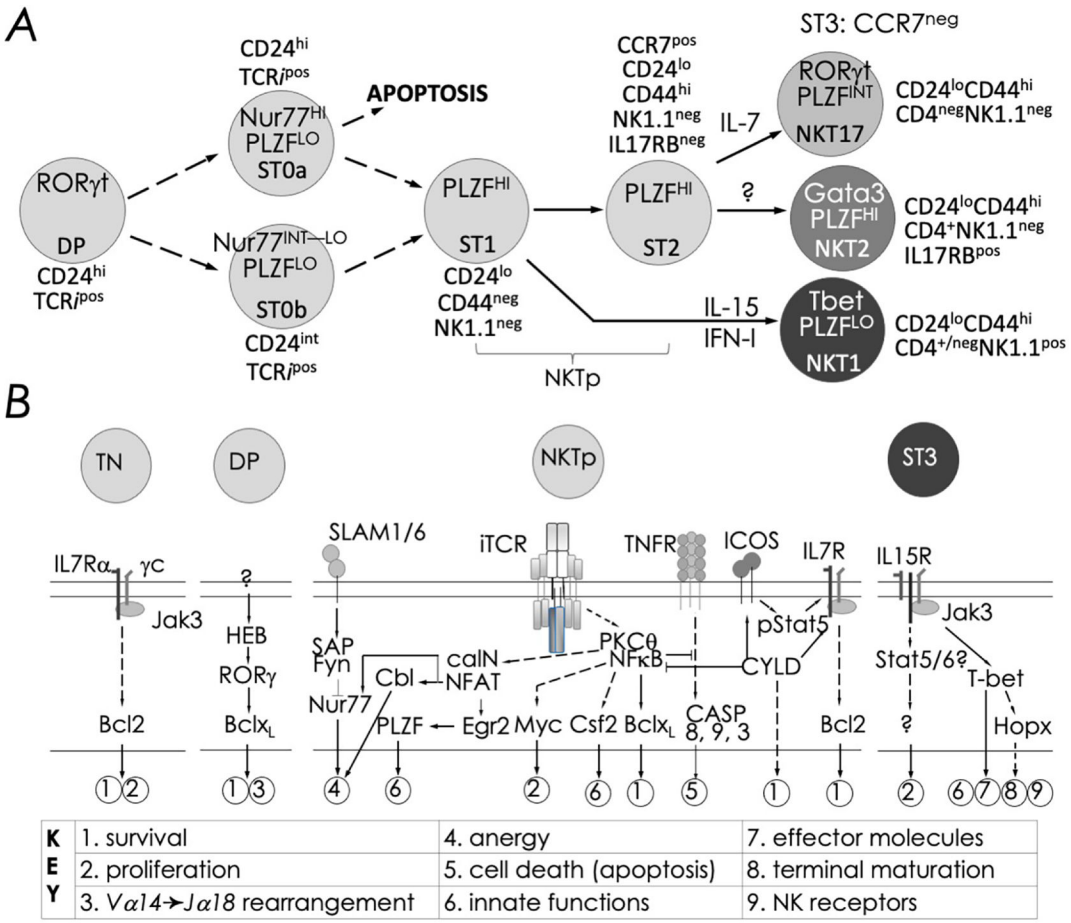


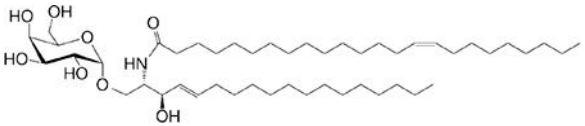
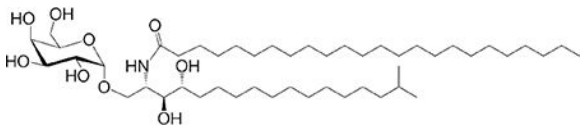
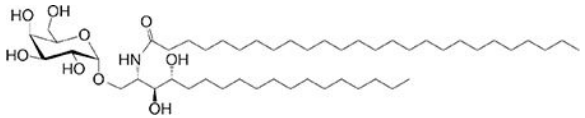
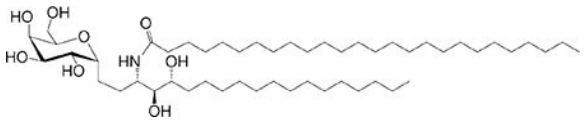
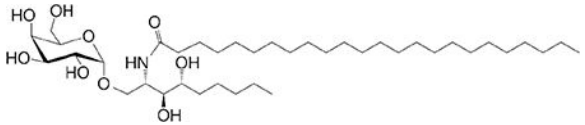
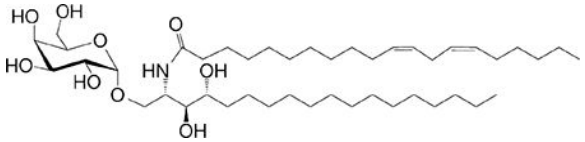
FIG. 7:

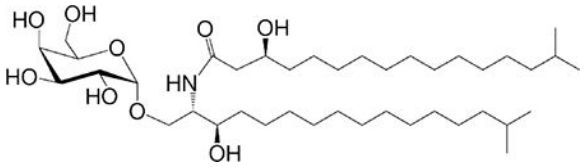
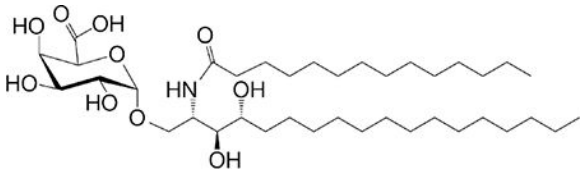
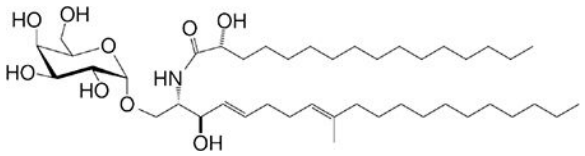
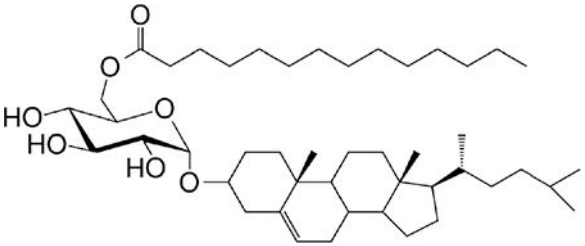
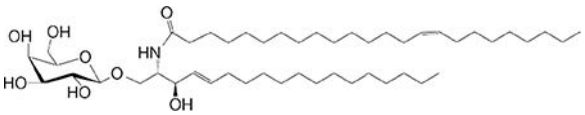
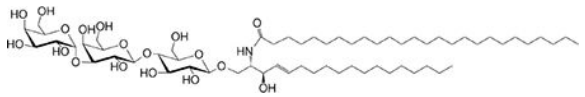
Schematic rendition of developmental stages and signaling events essential to NKT cell ontogeny: (A) Precursor ST0, immature ST1 & ST2, and mature ST3 represent distinct NKT cell developmental stages (ST) and NKT1, 2 and 17 are functional subsets. NKT cell ontogeny begins with the rearrangement of the *TRAV11*02* to *TRAJ18* TCR α-chain gene segments and proceeds after its interaction with the positively selecting CD1d-self lipid complex. Stage-specific NKT cell markers —e.g., CD24, CD44 and NK1.1—and subset-specific differentiation signals and transcription factors are indicated. Interleukin (IL)-7 and IL-15 are cytokines that mediate intercellular communication. (B) Thymocyte development to DP stage are essential for conventional $CD4^+$ and $CD8^+$ T as well as NKT cell development. Commitment to the NKT cell lineage occurs with the semi-invariant TCR expression at the DP stage under the influence of HEB (an E2A family transcription factor)- and RORγt. Positive selection at stage 0 induces the expression of PLZF, distinguishing NKT cells from other T lineages—e.g., $CD4^+$ and $CD8^+$ T cells. Onward development requires signals relayed via the lymphocyte activation molecule family member (SLAMF)-SLAM-associated protein (SAP)-Fyn (a Src kinase) module, which tempers agonistic signals relayed through the NKT cell TCR. NKT cell TCR signals are processed by protein kinase C (PKC)-θ and relayed to CBM (CARMA1-Bcl10-MALT1) complex for integration by the transcription factor nuclear factor-κB (NF-κB). Signals integrated by the SLAM-SAP-Fyn and the NKT cell TCR-PKC-θ-CBM-NF-κB modules culminate in *Gata3* and *Tbx21*,

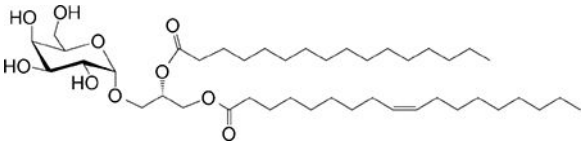
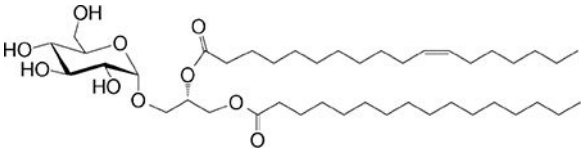
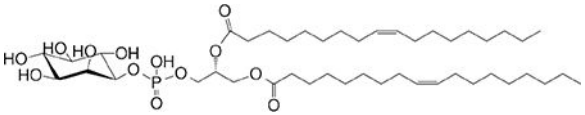
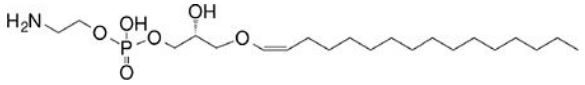
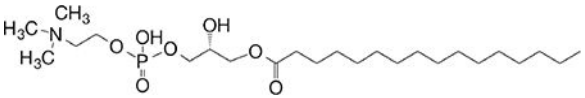
encoding T-bet. The two transcription factors are essential for *Il4* and *Ifng* transcription and lineage specific cytokine production. Calcineurin-NFAT-Egr2 and PKCθ-NF-κB signaling axes play essential roles in lineage proliferation (e.g., Myc), maintenance (e.g., Bcl-xL), and effector differentiation (e.g., Csf2), and maturation. Whilst the mechanism/s of subset differentiation are still debated, signals from IL-7 direct NKT17 cell differentiation and those from IL-15 and/or IFN-I (type I IFN) direct NKT1 cell differentiation. Transcription factors such as Egr-2, Ets-1, GATA3, Id2, Id3, MEF, Nur77, RORγt, and T-bet act at distinct stages shown to direct proper, functional NKT cell development. See text for details and references therein.

TABLE 1:

Synthetic, microbial, and self NKT cell agonists—structures and properties (adapted from refs [10,294])

Lipid (class[1]) biologic vs. synthetic	Chain length[2]	Structure	Response[3]	Refs.
αGalCer (GSL) self	C18 C24:l		IFN-γ, IL-4	56
Agel 9b (GSL) *Agelas mauritianus*	C17 (C_{16}-Me) phyto C24		Anti-tumor; *Agelas mauritianus*	286,287
KRN7000 αGalCer (GSL) synthetic	C18-phyto C26		IFN-γ IL-4 and other cytokines	236
α*C*Gal-Cer (GSL) synthetic	C18-phyto C26		Weak (mo[4])-to-none (hu); IFN-γ	288
OCH (GSL) synthetic	C9-phyto C24		Weak (mo)-to-none (hu); IL-4 (low-to-no IFN-γ)	115
C20-diene (GSL) synthetic	C18-phyto C20:2		IL-4 (low-to-no IFN-γ)	114

Lipid (class[1]) biologic vs. synthetic	Chain length[2]	Structure	Response[3]	Refs.
αGalCer (GSL) *Bacteroides fragilis*	CI7-C_3OH C17		Stimulatory and inhibitory based on sphanganine branching	27,33,289
αGalU Cer (GSL) *Sphingomonas* spp.	C18-phyto C14		Weak IFN-γ	30–32
Asp B (GSL) *Aspergillus fumigatus*	C20:2-C_9 Me C16-C_2 OH		Weak agonist	26
αGlc-6-acyl-Chol *Helicobacter pylori*	C14		Activates a small NKT cell subset (mo)	34
βGalCer (GSL) self	C18 C24:l		Weak agonist	290,291
iGb3 (GSL) self	C18-C24		Weak (mo)-to-no (hu) response	255

Lipid (class[1]) biologic vs. synthetic	Chain length[2]	Structure	Response[3]	Refs.
αGal-DAG (GGL) *Borrelia burgdorferi*	*sn1*-ClZA *sn2*-C16		Weak agonist (mo)	29
αGlc-DAG (GGL) *Streptococcus pneumoniae*	*sn1*-C18:1 *sn2*-C16		Weak agonist	28
PtdIno (GPL) self	*sn1*-C18:1 *sn2*-C18:1		Week agonist (mo)	59,292
Plasma-logen (GPL) self	*sn1*-C16 vinyl-ether *sn2*-lyso		Agonist for positive selection (mo)	293
Lyso-PtdCho (GPL) self	*sn1*-C16 *sn2*-lyso		Weak (hu)-to-no (mo) GM-CSF (no IL-4, IFN-γ) response	58

[1] Agel, agelasphin; Asp B, asparamide B; Chol, cholesterol; DAG, diacylglycérol; GalCer, galactosylceramide; GalUCer, galacturonosylceramide; GlcCer, glucosylceramide; PtdCho, phosphatidylcholine; PtdIno, phosphatidylinositol; *sn,* stereo nomenclature for glycerolipids GGL, glycoglycerolipid; GPL, glycerophospholipid; GSL, glycosphingolipid

[2] Sphingosine/phytosphingosine chain length indicated first and *N*-acyl chain length second

[3] Agonist strength based on ref[295]

[4] mo, mouse; hu, human

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