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Metabolic Triggers of Invariant Natural Killer T-Cell Activation during Sterile Autoinflammatory Disease

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Abstract

Ample evidence exists for activation of invariant natural killer T (iNKT) cells in a sterile manner by endogenous ligands or microbial antigens from the commensal flora, indicating that iNKT cells are not truly self-tolerant. Their controlled autoreactivity state is disturbed in many types of sterile inflammatory disease, resulting in their central role in modulating autoimmune responses. This review focuses on sterile iNKT-cell responses that are initiated by metabolic triggers, such as obesity-associated inflammation and fatty liver disease, as a manifestation of metabolic disease and dyslipidemia, as well as ischemia reperfusion injuries and sickle cell disease, characterized by acute lack of oxygen and oxidative stress response on reperfusion. In the intestine, inflammation and iNKT-cell response type are shaped by the microbiome as an extended “self.” Disease- and organ-specific differences in iNKT-cell response type are summarized and help to define common pathways that shape iNKT-cell responses in the absence of exogenous antigen.

Keywords

natural killer T cell; sterile inflammation; autoreactivity; metabolism

I Introduction

During his long and productive career, Dr. Eli Sercarz taught us the importance of self–nonself discrimination in adaptive immune response. His work emphasized how peptide antigenic determinants or epitopes can govern immune response character and how epitopes influence self-tolerance and autoimmunity.^{1–3} Because T-cell receptor (TCR) $\alpha\beta^+$ cluster of differentiation (CD)4 and CD8 T lymphocytes are positively selected by self-peptides in thymus, the distinction between self and nonself is not absolute. All T lymphocytes, at least those with $\alpha\beta$ T-cell antigen receptors (TCRs), have some measure of self-reactivity. Moreover, specialized populations of T lymphocytes require a higher TCR signal for their differentiation, referred to as agonist selection.⁴ Such cells are more overtly self-reactive in the periphery, but this self-reactivity is apparently controlled by inhibitory receptors and

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other means. Agonist-selected T-cell populations are considered to be innate-like, often described as existing at the interface of innate and adaptive immune responses. This review focuses primarily on one population of agonist-selected, innate-like T lymphocytes: type-I or invariant natural killer T (λ NKT) cells.

II Sterile Inflammation

Considering the issue of self-reactivity, we discuss innate-like T cells and sterile inflammatory pathways, with a focus on λ NKT-cell activation in sterile autoinflammatory diseases of the lung, liver, adipose tissue (AT), and gut. Sterile inflammation denotes an immune response that is mounted in the absence of an invading pathogen or microbial stimulation but in response to a noxious stimulus that results in tissue damage and inflammation. This stimulus can be induced acutely, for example, by mechanical, chemical, or thermal insult, or it can be chronic, when an underlying defect causes the long-term presence of inflammatory triggers. In either case, the immune system has an array of mediators and sensors that relay this information to activate inflammatory response pathways. Of note, similar triggers and sensors can simultaneously activate pathways to counteract inflammation by promoting resolution and tissue-repair pathways. This results in a coordinated and complex inflammatory healing response, first characterized by innate immune effectors and ultimately resulting in inflammatory response modulation and resolution to repair the tissue injury. Many of these responses have been evolutionarily designed to fight invading pathogens in the absence of adaptive immunity, which may explain why they often result in exacerbated tissue injury rather than efficient healing during sterile inflammation. This highlights the importance of innate-like T cells, which have the power to react quickly and the flexibility to modulate immune responses with a diverse set of effector qualities.

III Innate-Like T Cells

Adaptive immunity has long been appreciated as a main pathway through which the body eliminates invading pathogens with high specificity. B and T cells are the two types of adaptive immune cells. B cells produce highly specific antibodies that can bind to linear or conformational epitopes on proteins or to carbohydrates or lipids to neutralize pathogens. T-cell epitopes are short sequences presented by major histocompatibility complex (MHC)-encoded antigen-presenting molecules. In each case, the requirement is a massive expansion of the rare clones with correct specificity for the invading pathogen, thus leading to a delay in kinetics for the typical adaptive immune response. Bridging this gap between immediate responders and adaptive immunity, the immune system uses innate-like or nonconventional lymphocytes that have crucial roles as rapid responders, albeit with a greatly decreased repertoire of specificities.⁵ Unconventional T cells include T lymphocytes that recognize nonpeptide antigens that, in some cases, are presented by monomorphic antigen-presenting molecules. Members of this family include various $\gamma\delta$ T cells, including a population prevalent in human blood that express a V γ 9V52 TCR that recognizes pyrophosphate-containing compounds bound to butyrophilin proteins. The family also includes mucosal-associated invariant T (MAIT) cells that recognize metabolites of the vitamin B pathway presented by an MHC class I-related gene protein (MR1) as well as λ NKT cells that respond

to lipid antigens presented by CD1d.⁶ Unique features that define this group of cell types include (1) their memory-like or, at least, antigen-experienced phenotype, shown by expression of cell-surface proteins such as high amounts of CD44; (2) in the case of MAIT and λ NKT cells, conserved specificity throughout evolution⁷ and previous antigen experience as a part of their maturation; and (3) their poised and ready-to-respond state, enabling rapid cytokine responses within hours that can also occur in the absence of TCR stimulation, for example, via interleukin (IL)-12 and IL-18 exposure.

Because these features place the cells at the boundary of adaptive and innate immune systems, they also play a key part in inflammatory responses that occur in the absence of a foreign pathogen. The roles of MAIT, $\gamma\delta$ T cells, and other innate lymphocytes in self-reactivity have been reviewed elsewhere,^{8,9} so we focus on pathologies and mechanisms whereby λ NKT cells contribute to sterile inflammatory responses and inflammation when no foreign antigen or only commensal bacteria are present.

IV λ NKT Cells

λ NKT cells are highly conserved innate-like T lymphocytes that express invariant TCR α chains (V α 14-J α 18 or *Trav11-Traj18* in mice and V α 24-J α 18 or *Traj10-Traj18* in humans) and are paired with a restricted diversity of TCR-V β chains, predominantly V β 2, 7, or 8 in mice and V β 11 in humans. They recognize glycolipid antigens that are presented by CD1d, a nonpolymorphic MHC class I-like antigen-presenting molecule. Activation can occur in response to microbial glycolipid antigens or tissue injuries that trigger self-lipid antigen production to produce sterile inflammation.¹⁰ Activation can be amplified or even entirely dependent on cytokines from innate immune cells, mainly IL-12 and -18, and directly augmented by danger-associated molecular patterns (DAMPs) such as IL-33 or high-mobility group box 1.^{10,11} λ NKT cells can stimulate immunity and inflammation, although anti-inflammatory responses have also been described. These surprisingly opposing outcomes may be mediated in part by selective activation of one of several distinct functional subsets that express signature transcription factors (TFs) and produce specific cytokines. Several of these λ NKT-cell subsets are similar to MHC class II-restricted CD4⁺ T helper (T_H)1, 2, and 17 cells.¹² An important difference, however, is that λ NKT cells mostly differentiate into these divergent subsets in the thymus and when exogenous antigenic stimulation is absent.¹³ For reasons that are incompletely understood, when λ NKT cells exit the thymus and seed peripheral organs, a clear preference occurs in the location of λ NKT-cell subsets in different organs.¹⁴ In the following sections, we discuss features of λ NKT-cell responses that occur in different organs, with respect to how they control sterile inflammation and autoinflammatory pathology outcomes that are caused by metabolic triggers.

V Metabolic Triggers for λ NKT Cells

Many types of sterile inflammation involve a metabolic trigger as the central pathology pathway, and in each case, evidence exists for the critical role of λ NKT cells in modulating immune response. We discuss examples including (1) fatty liver disease as a manifestation of metabolic disease and dyslipidemia, where λ NKT cells have opposing roles at different

disease stages; (2) ischemia reperfusion injury (IRI), caused by acute lack of oxygen and an oxidative stress response that is initiated on reperfusion (with IRI in both liver and lung, λ NKT activation mainly leads to pathogenesis via inflammatory cytokine excretion); (3) sickle cell disease (SCD), a chronic condition of repeated IRI, wherein λ NKT cells are proinflammatory; (4) obesity-associated inflammation, with a balance of enriched immune-regulatory λ NKT cells in AT that is perturbed during metabolic disease; and (5) intestinal inflammation, in which λ NKT-cell responses are shaped by the microbiome as extended “self.”

VI The Fat, The Liver, and The Fatty Liver

A The Fat

Consistent with their role in detecting and responding to lipid molecule changes, λ NKT cells play a central part in the immune system in AT and inflammation that is associated with obesity and insulin resistance (IR).¹⁵ In AT, λ NKT cells are enriched in comparison to other organs and comprise up to 20% of resident T cells.^{16,17} The phenotype of AT λ NKT cells is most consistent with λ NKT2/ λ NKT10 immunoregulatory function, because they readily produce IL-4 and -10 in higher amounts than in most other organs.^{17–19} Accordingly, during obesity chronic inflammation leads to reduced λ NKT-cell numbers, and the capacity to produce IL-4 diminishes when inflammation developed.^{16,17} Genetic deletion either of adipocyte or macrophage expression of CD1d or λ NKT cells, by deletion of the required Ja segment, left mice susceptible to high-fat diet (HF-D)-induced obesity, inflammation, and IR.^{16–18} This coincided with loss of λ NKT-cell-derived IL-4, lack of arginase I induction, and M1 inflammatory macrophage polarization.^{16,20} Exacerbated obesity-associated inflammation and weight gain are reversed by λ NKT-cell adoptive transfer.¹⁶ However, reports also describe an inflammatory phenotype of λ NKT cells during obesity-associated inflammation,²¹ and accordingly, these improved disease via Cd1d deletion,²² perhaps suggesting opposing roles of λ NKT cells at different stages of obesity-induced disease. A stage-specific opposing effect of λ NKT-cell activity has been described during atherosclerosis, in which λ NKT-cell proliferation and IL-4 production were atheroprotective in early stages of asymptomatic plaques.²³ But, as cholesterol depositions progress, λ NKT cells become well-established drivers of pathology through proinflammatory cytokine production within atherosclerotic lesions.²⁴

B The Liver

Among all organs, liver contains the highest frequency of λ NKT cells, which are enriched by 20-fold or more in mice.²⁵ Within this comparatively large λ NKT-cell population, a clear bias exists toward the λ NKT1 phenotype, evident by both phenotypic analysis and predominant interferon (IFN)- γ production in response to activation.²⁶ In the context of sterile hepatic disease, λ NKT activation may be mediated by diverse antigen-presenting cells (APCs) with high levels of CD1d expression on liver sinusoidal endothelium, Kupffer cells, hepatocytes, and stellate cells.¹⁴ Unsurprisingly, administration of the highly potent glycolipid agonist α galactosyl ceramide (α GalCer) led to efficient antigen presentation and hepatic λ NKT-cell activation, resulting in severe pathology and lethal inflammation.²⁷ Although this demonstrates the power and danger of augmented hepatic λ NKT responses,

administration of a strongly activating synthetic glycolipid antigen does not fully represent human sterile inflammatory disease physiology in the liver.

C The Fatty Liver

Nonalcoholic fatty liver disease (NAFLD) is a sterile autoinflammatory disorder that has become a major health problem, particularly in developed countries, where up to 30% of adults may be affected.²⁵ Disease can progress to nonalcoholic steatohepatitis (NASH) with fibrosis and cirrhosis, driven by obesity and abnormal accumulation of fat in the liver.²⁶ Analyses of leptin-deficient *ob/ob* mice^{28–30} and mice on an HFD^{31–33} or choline-deficient diet^{34,35} have demonstrated that diet or genetically induced obesity can lead to decreased hepatic IL-12, diminished hepatocyte CD1d expression, and impaired lipid presentation that are ultimately associated with loss of hepatic λ NKT cells in fatty livers.²⁶ On a related note, transferring λ NKT cells into obese mice reduced inflammation and fatty liver,³⁶ and deletion of λ NKT cells in *Ja18*-deficient mice exacerbated fat accumulation,¹⁶ thus arguing for a protective role of λ NKT cells in NAFLD. Of note, AT is enriched in λ NKT10 cells, and this may contribute to anti-inflammatory pathways as liver accumulates fat.¹⁶ Other studies, however, reported that hepatic steatosis-induced endoplasmic reticulum (ER) stress led to reduced Cd1d expression and dysregulated λ NKT-cell responses in liver³⁰ but ameliorated hepatic steatosis in *Cd1d*^{-/-} or *Ja18*^{-/-} mice.^{21,37} This may be explained by pathogenic λ NKT-cell accumulation at later stages of NASH.³⁸

It appears that a shifting balance of λ NKT1-, 2-, and 10-cell subsets or responses at different stages is a critical determinant in the liver-damage setting due to metabolism-induced inflammation. The primary trigger of this pathology is hepatic manifestation of a metabolic syndrome, caused by severe chronic changes in a systemic metabolic state. Given metabolism's central role in T- and λ NKT-cell subset polarization and function,³⁹ hallmarks of NAFLD such as dyslipidemia and hyperglycemia may be intrinsic and causative pathways for profound changes in λ NKT-cell populations. As disease progresses, the metabolome changes, which influences λ NKT response. In light of this, balance modulators of λ NKT-cell responses may be (1) amount and type of antigenic lipids (self or derived from food intake or the microbiome) that accumulate; (2) changes in APC self-lipid antigen presentation and cytokine production; (3) induced λ NKT10 fate in fat tissue; and (4) how liver λ NKT1/2 balance is biased by the altered metabolic environment.

D The Hypoxic Liver

Another type of hepatic sterile inflammation is liver IRI, a condition that results from interruption and then reinstatement of an organ's oxygen supply. IRI occurs during hypoxic hepatitis, liver resection surgery, and liver transplantation. A rising number of liver transplantations for NAFLD, among other diseases, requires the use of marginal organs, but that is hindered by IRI.²⁶

Properties of λ NKT cells suggest that they could be central players in liver IRI²⁵ because (1) liver has a high degree of λ NKT-cell prevalence; (2) rapidly developed liver IRI is inconsistent with the time frame required for conventional T-cell responses and rapid cytokine production; and (3) in mice, lymphocyte expansion during reperfusion following a

hypoxic episode is largely comprised of λ NKT cells.⁴⁰ Indeed, during murine hepatic IRI, λ NKT cells contribute to tissue damage via IFN- γ production.⁴¹ In λ NKT-cell-deficient mice or in response to anti-CD1d monoclonal antibodies or λ NKT-cell-depleting antibodies, damage is reduced.⁴² Furthermore, introduction of λ NKT cells by transfer into *Rag1*^{-/-} mice restores pathology.⁴¹ This generally argues for a pathological role of λ NKT cells that mediate an λ NKT1 inflammatory response following IRI. In line with this observation, treatment with a synthetic adenosine 2A receptor (A_{2A}R) agonist that reduced λ NKT-cell IFN- γ production led to significant liver damage amelioration and a 60% reduction in serum alanine aminotransferase levels.⁴¹

Although λ NKT-cell activation drives liver damage, compensatory mechanisms such as induction of A_{2A}R signaling during hypoxia are in place to limit organ pathology.⁴³ The family of hypoxia-inducible TFs (HIFs) has crucial roles in T-cell survival and function, and HIF2 α -deficient mice developed more severe IRI that was mediated by λ NKT cells.⁴⁴ Ischemic preconditioning, a well-known phenomenon, describes mild hypoxia pre-exposure that induces such anti-inflammatory pathways.⁴⁵ Inducing A_{2A}R expression by hypoxia and ameliorating IRI by preconditioning with α GalCer⁴⁶ are two pathways that counteract excessive λ NKT-cell activation. In this context, α GalCer treatment induced λ NKT-cell A_{2A}R expression and attenuated hepatic IR injury via IL-13.⁴⁶ Similarly, deleting the oxygen sensor prolyl hydroxylase family in T cells constitutively activated HIF signaling and protected against hepatic IRI,⁴⁷ but whether this effect is mediated by λ NKT cells remains to be shown.

VII λ NKT Cells at Mucosal Surfaces: Lung and Intestine

A Lung

Because the underlying trigger during IRI is transient cessation of tissue oxygen supply followed by reoxygenation, one may expect similar pathologic mechanisms with IRI in organs other than liver. Lung is the principal organ of gas exchange for release of carbon dioxide and uptake of oxygen into the blood through fine capillaries surrounding alveolae. Lungs are prone to IRI, a major complication causing graft failure during lung transplantation. In mouse lung, λ NKT cells constitute ~ 5% of resident lymphocytes, with λ NKT1 and 2 predominantly in vasculature and λ NKT17 in tissue.¹⁴ λ NKT17 cells are a prevalent subset in lungs, with comparatively large amounts of IL-17 produced in response to activation with α GalCer, leading to neutrophil recruitment into bronchoalveolar lavage fluid^{48,49} and subsequent tissue damage.

B The Hypoxic Lung

1 Ischemia Reperfusion Injury—Neutrophil recruitment is a pulmonary IRI hallmark, and although IFN- γ from λ NKT cells is implicated in liver, IRI in lung is mediated by λ NKT-cell production of IL-17.⁵⁰ Similar to liver IRI, pulmonary adenosine receptor expression that is induced by hypoxia is a protective response mechanism limiting λ NKT-cell-mediated injury.⁵¹ Activation of nicotinamide adenine dinucleotide phosphate oxidase (NOX)2 and a burst of IRI-associated oxidative stress occurs in λ NKT cells and is repressed by adenosine signaling.⁵² Consequently, NOX2 deletion or adenosine receptor agonist treatment prevents

IL-17 production by murine and human λ NKT cells after acute hypoxia reoxygenation in wild-type mice.⁵² This defines a novel signaling mechanism of IL-17 production by λ NKT cells mediated by NOX2 activation after lung IRI.

2 Sickle Cell Disease—In SCD, misshapen erythrocytes evoke widely disseminated transient microvascular IRI. Resulting vaso-occlusion is the pathogenic basis for the most common morbidity in SCD: acute chest syndrome. In lung, a profound inflammatory response occurs, characterized by leukocyte activation, migration, and adhesion.^{53,54} Among leukocytes, pulmonary λ NKT cells increased in number and became activated in mice bearing the hemoglobin β gene mutation that is characteristic of SCD (called SCD mice).⁵⁵ Lung λ NKT cells displayed significantly increased expression levels for the activation marker CD69 and IFN- γ , whereas interruption in λ NKT-cell activation or migration into lung reduced pulmonary inflammation and improved pulmonary function in SCD mice.⁵⁵ SCD patients have had elevated λ NKT cells in blood, expressing higher levels of activation marker CD69 and elevated C-X-C motif chemokine receptor 3 (CXCR3).⁵⁵ As with other models of lung IRI, adenosine agonists reduced λ NKT-cell activation and limited tissue damage in mouse lungs.⁵⁶

C The Intestine

The intestine is another example of a mucosal surface that centrally involves λ NKT cells during both homeostasis and inflammatory bowel disease. By constant exposure to diverse dietary products and a dense microbiome, λ NKT cells are uniquely challenged to coexist with and contain commensal growth and avoid unwarranted damage to intestinal tissue. Thus, although the intestinal mucosal environment is far from sterile, the immune system normally tolerates the microbiome, which can therefore be seen as “extended self,” with a central role in preventing autoimmune disease through interaction with λ NKT cells and other cell types. At homeostasis, up to 3% of intestinal $\alpha\beta$ T cells are λ NKT cells, with highest frequency in small intestine lamina propria, and their numbers are inversely correlated with bacterial abundance.⁵⁷ In germ-free (GF) mice, higher numbers of λ NKT cells locate to the intestine. They can contribute to increased autoinflammatory diseases of intestine and lung, for example, in colitis that is induced by the hapten oxazolone.^{57,58} Interestingly, inflammation was improved after neonatal GF mice exposure to bacteria, suggesting that bacterial antigens may shape the mucosal λ NKT-cell phenotype early in life.⁵⁹ Indeed, evidence for presentation of microbial antigens to λ NKT cells stems from high Nur77 expression in intestinal λ NKT cells, a marker for recent TCR stimulation.⁶⁰ *Sphingomonas* species and *Bacteroides fragilis*, among others, were found to be sources of antigenic lipids that stimulated λ NKT cells. This cross-talk is CD1d dependent, because myeloid CD1d expression loss alters the intestinal λ NKT cytokine profile.⁶⁰ Conversely, λ NKT-cell loss alters microbiome composition.⁶¹ Whereas bone-marrow-derived CD1d signals contribute to λ NKT-cell-mediated intestinal inflammation, engaging epithelial CD1d elicits protective effects by activating signal transducer and activator of transcription 3-dependent IL-10 transcription.⁶²

D The Inflamed Intestine

In a T_H2 cytokine-driven model of colitis induced by oxazolone, λ NKT cells were pathogenic, accumulated in the gut, and drove inflammation that was mediated by IL-13.⁶³ Conversely, mice were protected when CD1d was blocked with antibodies, when the gene encoding CD1d was deleted, or in *Ja18*^{-/-} mice, underlining the pathogenic role of λ NKT cells in this model.^{58,63} On the other hand, Crohn's disease λ NKT cells possessed a proinflammatory cytokine signature.⁶⁴ During dextran sulfate sodium (DSS)-induced colitis, λ NKT cells were protective only when preactivated by antigen exposure.⁶⁵ These opposing DSS model outcomes may be explained by differences in microbiota that may shape λ NKT cells and inflammatory response.

VIII Mechanisms of λ NKT-Cell Activation

In reviewing the literature on sterile inflammation and autoinflammatory disease, mouse λ NKT cells were reported to be key players in each organ discussed above, but pathogenic versus beneficial functions were highly situation and organ specific and could change during disease progression. Specific λ NKT-cell subset dominance may partly control opposing outcomes.¹⁴ A crucial element is the pathway responsible for λ NKT-cell activation and whether in some cases true autoreactivity, meaning recognition of self-antigens presented by CD1d, caused the response. Evidence exists for several pathways that activate λ NKT cells in the absence of a microbial stimulus, and some of these may act in concert. These pathways are summarized in Fig. 1 and discussed below.

A Microbial Self-Lipids

In the intestine, lipid antigens that trigger immune pathology may be derived from naturally occurring microbial antigens. Glycosphingolipids, the most potent antigens, are found in *Bacteroidetes* species. In fact, the glycosphingolipid from the commensal organism *B. fragilis* is closely related to α GalCer, which although originally isolated from the marine sponge *Agelas mauritamus*⁶⁶ probably represents the product of a microbial colonist. *Sphingomonas* species are found in the environment and have antigenic glycosphingolipids such as α -glucuronosylceramide and α -galacturonosylceramide.^{67,68} Antigenic glycolipids are found in other commensal-associated bacteria such as *Streptococcus pneumoniae* and *Helicobacter pylori*.⁶⁹ These can be viewed as lipid antigens that compose part of "extended self."^{67,70} Of note, commensal microbes also populate lung, and microbial products are present in liver and may trigger λ NKT-cell responses during inflammatory disease. Apart from microbe-derived lipid antigens, λ NKT cells can also respond to self-lipids, including the β -linked glycosphingolipid isoglobotrihexosylceramide. Additional self-antigens for λ NKT cells have been reported, including for human λ NKT cells self-phospholipid antigens such as phosphatidylethanolamine, phosphatidylinositol, and phosphatidylcholine.^{70,71} The number of self-agonists for λ NKT cells and their relative importance remains to be determined. In addition, although λ NKT cells are agonist selected, the relationship between positively selecting self-antigens in thymus and self-antigens that stimulate them in the periphery is unknown. Regardless, to recognize diverse antigen molecules, the λ NKT TCR acts akin to a pattern recognition receptor, with an inherent affinity to CD1d as a molecular basis to detect and respond to diverse lipids in a relatively fixed orientation.^{72,73}

B Stimuli from Innate Cells

The ability of λ NKT cells to respond to inflammatory stimuli from innate cells, possibly in conjunction with self-glycolipid antigen recognition, points to redundancy as well as cooperation in mechanisms that activate λ NKT cells. Toll-like receptor signals increase synthesis of incompletely defined λ NKT-cell self-antigens.⁷⁴ Exposure to IL-12 and -18⁷⁵ or IL-12 and type-I IFNs⁷⁶ can be sufficient to activate λ NKT cells even in the absence of TCR signals, but this response is dominated by IFN- γ secretion. The same cytokines can also augment the response to self or microbial lipid antigens.

C Stress Pathways

Although relevant self-antigen structure(s) remain to be defined, recent findings provide insight into some signals that induce stimulatory presentation of autoantigens by APCs. A common feature of sterile autoinflammatory diseases is stress-associated signaling pathway activation, including the hypoxia response, ER stress response, autophagy, and oxidative stress response.⁷⁷ Activation of stress-induced signaling pathways in APCs could induce or facilitate self-lipid presentation or directly act in λ NKT cells to modulate their responsiveness or polarization toward effector phenotypes. Evidence for the former is based on recent findings that ER-stressed myeloid cells, via signaling events that were modulated by the protein kinase RNA-like ER kinase pathway, increased CD1d-mediated presentation of immunogenic endogenous lipid species, resulting in enhanced λ NKT-cell activation, both *in vitro* and *in vivo*.⁷⁸ Supporting evidence that ER stress is an important mechanism for eliciting endogenous CD1d-restricted λ NKT-cell responses was presented in a study reporting that neutral lipids from ER-stressed APCs shaped λ NKT-cell cytokine response.⁷⁹ Of note, hypoxia induces ER stress during ischemic injuries,⁸⁰ but whether this axis contributes to λ NKT-cell activation in IRI models remains unknown. In addition, we have not yet determined whether other stress-associated pathways or signals contribute to autoantigen presentation and CD1d-dependent activation of λ NKT-cell autoreactivity. Autophagy is an important stress-response pathway. Whereas autophagy-deficient APCs function equivalently to controls in antigen-presentation assays,⁸¹ autophagy reduced CD1d-mediated antigen presentation *in vivo* by increasing CD1d internalization.⁸² Hypoxia response is another important stress-response pathway, but whether it too modulates antigenic lipids that APCs present or affects λ NKT-cell responses directly remains to be shown. In addition, the role of DAMPs and alarmins in λ NKT-cell activation requires further investigation. Alarmin IL-33, for example, activates both mouse and human λ NKT cells independent of exogenous antigen.^{83,84} APC pretreatment with a number of metabolic modulators, including adenosine monophosphate-activated protein kinase and HIFs, augments λ NKT-cell hybridoma activation.⁸⁵ We do not know if these mechanisms also occur *in vivo*. Thus, APC metabolic changes can alter λ NKT-cell responses, but could the reverse also be true? One study describes APC uptake and neutral lipid content as augmented by coculture with λ NKT cells but not other T cells that were derived from mice with metabolic disease.⁸⁶ This mechanism depends on production of the tumor necrosis factor superfamily molecule tumor necrosis factor superfamily member 14 by λ NKT cells and reveals bidirectional cross-talk between λ NKT cells and their APCs, modulated by metabolic disease.⁸⁶ It remains to be shown, however, whether altered APC lipid metabolism also leads to new lipid-antigen presentation.

IX Summary

Taken together, ample evidence exists to show that λ NKT-cell activation in a sterile environment by endogenous ligands with or without cytokines form innate cells, indicating that λ NKT cells are not truly self-tolerant. Activation by microbial antigens from the commensal flora may also be considered a breach of strict self-tolerance. For example, their state of controlled autoreactivity is revealed by fulminant autoreactivity that is mediated by λ NKT cells. This results in mice in which all T cells are deficient for enzymes that demethylate cytosines in chromatin.⁸⁷ Thus, common molecular pathways that cause λ NKT-cell autoreactivity are being revealed. The quality of λ NKT-cell responses, such as cytokine profiles, subset composition, and dynamic changes in the context of sterile inflammatory disease, will help in providing further insights into how metabolic changes shape λ NKT-cell autoreactivity and the ways in which these processes may be altered for therapeutic benefit.

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Figure 1. Models of iNKT-cell activation pathways in sterile inflammatory disorders.

Common disease states that trigger autoreactive iNKT-cell activation include IRIs and metabolic diseases such as nonalcoholic fatty liver disease or obesity-associated inflammation. Several pathways may lead to activation and functional changes in iNKT cells in response to these stressors. First, APCs may sense stress directly and respond, for example, via the ER-stress-response pathway, to present self-lipid antigens and/or produce cytokines including IL-12 and -18. Each can trigger iNKT-cell activation. Second, the altered environment of stressed tissues may affect iNKT-cell subset composition indirectly, by sensing and responding to molecules that are released following tissue damage. IL-33 and high-mobility group box 1 are two examples of such stress-associated molecules that can modulate the iNKT-cell response. Third, metabolic stress signaling pathways can shape iNKT-cell subset differentiation and polarization directly, as has been widely demonstrated for HIF pathway genes, autophagy, and unfolded protein response.