Published in final edited form as:
Nat Rev Immunol. 2018 September ; 18(9): 559–574. doi:10.1038/s41577-018-0034-2.

Tissue-specific functions of invariant natural killer T cells

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Abstract

Invariant natural killer T cells (iNKT cells) are an innate-like T cell subset that expresses an invariant T cell receptor (TCR) α-chain and recognizes lipids presented on CD1d. They secrete diverse cytokines and can influence many types of immune responses. Despite having highly similar T cell receptor specificities, iNKT cells differentiate in the thymus into distinct subsets that are analogous to T helper 1 (T_H1), T_H2 and T_H17 cell subsets. Additional iNKT cell subsets that may require peripheral activation have also been described, including one that produces IL-10. In general, iNKT cells are non-circulating, tissue-resident lymphocytes, but the prevalence of different iNKT cell subsets differs markedly between tissues. Here, we summarize the functions of iNKT cells in four tissues in which they are prevalent, namely, the liver, the lungs, adipose tissue and the intestine. Importantly, we explain how local iNKT cell responses at each site contribute to tissue homeostasis and protection from infection but can also contribute to tissue inflammation and damage.

Invariant natural killer T (iNKT) cells were first described in the early 1990s as a mature T cell subset with a semi-invariant T cell receptor (TCR)[1,2]. This TCR comprises an invariant TCR α-chain (TCRα), which is formed by a conserved TCRα variable (*TRAV*) and TCRα joining (*TRAJ*) gene rearrangement. The TCR β-chain (TCRβ) is formed from a limited number of TCRβ variable (*TRBV*) segments, but it contains substantial diversity in its complementarity-determining region 3 (CDR3) owing to different variable β (Vβ)–diversity β (Dβ)–joining β (Jβ) gene rearrangements. These semi-invariant TCRs recognize lipids presented by CD1d, a non-polymorphic MHC class I-like antigen-presenting molecule[3–5]. CD1d-binding lipid antigens from various commensal and pathogenic microorganisms have been well characterized[6–9]; however, iNKT cells are also self-reactive, and several self-lipid antigens have been reported as well[6,9–12]. A number of additional properties distinguish iNKT cells from peptide-reactive TCRαβ^+ T cells. First, their specificity is highly conserved when different mammalian species are compared[13]. Second, they have a different developmental pathway in the thymus that includes positive selection by thymocytes instead of by cortical epithelial cells[14]. This pathway imparts an antigen-experienced phenotype and functional maturity to iNKT cells, even before they exit the thymus. Third, iNKT cells

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Author contributions
Both authors contributed to researching, writing and editing the manuscript.

Competing interests
The authors declare no competing interests.

respond very rapidly to TCR and/or cytokine signals with an immediate and copious production of cytokines. Such rapid responses allow iNKT cells to bridge innate and adaptive immune responses or even to participate in the innate response. Fourth, as discussed in more detail below, iNKT cells are tissue homing and tissue resident. Indeed, over the past 20 years, these cells have been found to play crucial roles in a variety of immune responses and pathological conditions (FIG. 1; TABLE 1)

Although iNKT cells play important roles in numerous lymphoid and non-lymphoid tissues, in this Review, we focus on four peripheral non-lymphoid tissue locations where these cells have been best characterized, namely, liver, lungs, adipose tissue and intestine (FIG. 1). We discuss the iNKT cell subset composition and sub-tissue location and the critical roles of iNKT cells within each tissue. We describe the complexity of the iNKT cell response and explain how, within the same tissue, iNKT cells can serve protective roles against harmful pathogens and contribute to tissue homeostasis yet also in some cases cause pathologic tissue damage. This could have considerable therapeutic implications. As CD1d is non-polymorphic, adoptive cell transfers using iNKT cells will not cause graft-versus-host disease. Therefore, the therapeutic use of stimulated or expanded iNKT cell populations, most recently as a platform for chimeric antigen receptors, is an area of active investigation[15–18] (BOX 1).

Functional subsets of iNKT cells

Thymic origin and post-thymic tissue localization.

In mice, iNKT cells differentiate in the thymus into at least three effector subsets, which resemble subsets of $CD4^+$ T helper cells and also subsets of innate lymphocyte cells (ILCs)[19–24]. Functional iNKT cell subsets can be distinguished on the basis of their expression of different cell surface markers and signature transcription factors (TABLE 2). For example, NKT1 cells are similar to T helper 1 (T_H1) cells and group 1 ILCs (ILC1s) in that they express similarly high amounts of the transcription factor T-bet (encoded by *TBX21*) and secrete IFNγ following activation. NKT1cells also show greater cytotoxic function than other iNKT cell subsets. A notable unique feature of NKT1 cells, which distinguishes them from T_H1 cells or ILC1s, is that they can produce IL-4 in addition to IFNγ when activated through their TCR. NKT2 cells secrete cytokines that include IL-4 and IL-13 and therefore resemble T_H2 cells, whereas NKT17 cells are similar to T_H17 cells with regard to their cytokine secretion profile (TABLE 2). In addition to showing differences in cytokine production, thymic iNKT cell subsets express distinct chemokine receptors and integrins, suggesting that they localize to different tissue sites after exiting the thymus.

In humans, functional subsets of iNKT cells are not as well defined as in mice, although there is evidence that iNKT cells in human blood that lack expression of CD4 and CD8 (double-negative (DN) iNKT cells) and those that express CD8 (either CD8αα or CD8αβ) are different from their $CD4^+$ iNKT cell counterparts[25–28]. The DN and $CD8^+$ iNKT cells found in humans were reported to be similar to mouse NKT1 cells, showing increased IFNγ secretion and cytotoxic function when activated[26,27]. Human iNKT cells have also been shown to be capable of producing IL-17 in a pro-inflammatory environment[29].

Some iNKT cells remain as long-term residents in the mouse thymus[30], where they provide important homeostatic functions. Homeostatic IL-4 production by these cells, which likely belong to the NKT2 cell subset[30], influences the differentiation of so-called 'natural memory' eomesodermin (EOMES)-expressing $CD8^+$ T cells. These mature $CD8^+$ thymocytes have an antigen-experienced phenotype typified by high levels of expression of CD44 and CD122 (also known as IL-2Rβ)[31,32]. IL-4 secretion by thymic iNKT cells also acts on medullary epithelial cells to allow for normal egress of mature, MHC class II-restricted $CD4^+$ T cells[33]. After they egress from the thymus, iNKT cells are largely non-circulating long-term residents in several lymphoid and non-lymphoid tissues, including the liver, lungs, spleen, lymph nodes, intestine, adipose tissue and bone marrow, as shown by parabiosis experiments in mice[34,35] (FIG. 1). There is limited understanding of what recruits iNKT cell subsets to different tissues. Within peripheral sites, iNKT cells largely do not require TCR stimulation for homeostatic maintenance and long-term survival[36–38], a property they share with memory T cells[39,40]. However, while iNKT cells are present in mice lacking peripheral CD1d expression, some recent studies have indicated that CD1d in the periphery is important for their full maturation; for example, expression of NK1.1 or in some tissues peripheral CD1d influences subset distribution or regulates apoptosis[37,41,42]. Similarly to memory $CD4^+$ and $CD8^+$ T cells, the homeostasis of peripheral iNKT cells depends on cytokines, with NKT1 cells dependent on IL-15 (REFS[36,43]). By contrast, survival and expansion of NKT17 cell populations is dependent on IL-7 (REF.[44]).

Different iNKT cell subsets are enriched in distinct tissues (FIG. 1; TABLE 2). For example, NKT1 cells are highly enriched in the mouse liver[45], while NKT17 cells are located primarily in lymph nodes, skin and the lungs, with a minor population also present in the spleen[45]. NKT2 cells populate several sites including the lung and spleen, but they are uniquely more abundant in mesenteric lymph nodes[45]. While not discussed in this Review, iNKT cells are also rapidly activated in peripheral lymph nodes and may play critical roles in anti-pathogen responses[46–48]. Notably, the overall subset frequencies also vary between different mouse strains. For example, NKT1 cells are the most abundant subset in C57Bl/6 mice, while BALB/c mice and several other inbred strains have more NKT2 cells[45].

Post-thymic induction of iNKT cell subsets.

Although NKT1, NKT2, and NKT17 cell subsets differentiate in the thymus, there is evidence suggesting that marked post-thymic differentiation of effector iNKT cell subsets also occurs in both mice and humans[28,49,50]. In mice, expression of the natural killer (NK) receptor NK1.1, a hallmark of mature NKT1 cells in the thymus, was not found on recent thymic emigrants (RTEs) but was induced when the cells were exposed to CD1d in the periphery[49,50]. Similarly, human thymic iNKT cells express low levels of CD161, a member of the NKR-P1 family related to mouse NK1.1, but they express high levels upon maturation in the periphery[51,52]. Furthermore, using intra-thymic fluorescein isothiocyanate (FITC) injection in mice to label iNKT cell RTEs, these cells were found to be not only $NK1.1^-$ but also more proliferative, and upon in vitro activation, they secreted more IL-4 (REF.[50]). In the RTE population, a higher percentage also expressed neuropilin 1 (NRP1) and produced IL-17 (REF.[50]). These data indicate that the iNKT cell RTEs are not representative of the

mature iNKT cell populations found in the thymus, liver or spleen at steady state, suggesting that they are less mature and subject to further differentiation signals in the periphery.

Follicular helper NKT (NKT_{FH}) cells have been described, and they are similar to T follicular helper (T_{FH}) cells that act in germinal centres to promote affinity maturation of antibodies[53,54] (BOX 2). IL-10-producing NKT (NKT10) cells that resemble type 1 regulatory T cells have also been characterized[34,55,56]. These iNKT cell populations have been identified in the periphery, with NKT10 cells highly enriched in adi-pose tissue[34,55,56] and NKT_{FH} cells primarily localized in the spleen, but they have not been found in the thymus of wild-type mice. However, in analyses of TCRβ mutant mice that have a β-chain substitution affecting TCRα and TCRβ pairing, IL-10-producing iNKT cells were found in the thymus and were increased in adipose tissue[57]. These data suggest that the quality of TCR signalling in the thymus influences the frequency of these cells. In wild-type mice, both NKT_{FH} and NKT10 cells expanded after immunization with highly potent glycolipid antigens, either owing to proliferation of rare precursors or conversion from other subsets[34,53,56]. Notably, NKT10 cells were transcriptionally and functionally distinct from other iNKT cell subsets and had a regulatory phenotype. Promyelocytic leukaemia factor (PLZF) is a transcription factor that is critical for the differentiation of iNKT cells and their acquisition of effector functions, and various amounts of PLZF are expressed in mature iNKT cell subsets. NKT10 cells were an exception, however, as they expressed little or no PLZF, and instead they expressed E4 promoter-binding protein 4 (E4BP4; also known as NFIL3), a transcription factor that regulates expression of IL-10 (REF.[58]). Additional functions have been attributed to iNKT cells, including the secretion of IL-9 (REFS[59,60]) and IL-22 (REFS[61,62]), even in the absence of IL-17 secretion[63]. Finally, *FOXP3* expression by iNKT cells has been reported following their exposure to transforming growth factor-β (TGFβ)[64].

Activation by antigen and cytokines.

Within different tissues, iNKT cells can be activated by CD1d-mediated presentation of foreign or self-antigens, which can be augmented by cytokine stimulation. They can also be activated by cytokines when TCR stimulation is absent, at least for NKT1 cells exposed to lipopolysaccharide (LPS) or IL-12 in combination with other cytokines, such as IL-18 (REFS[65,66]). A similar, cytokine-dependent activation of human iNKT cells has been reported[67], although these cells may have recently undergone TCR-dependent stimulation owing to CD1d presentation of self-antigens[68]. When stimulated by cytokines in the absence of concomitant TCR stimulation, iNKT cells produced IFNγ, but they did not secrete other cytokines normally induced by the TCR, such as IL-4. TCR stimulation led to rapid, robust secretion of a variety of cytokines, which induced the activation of most other haematopoietic cells, including iNKT cells, NK cells, macrophages, dendritic cells (DCs), B cells and T cells. Which cytokines are prevalent is influenced by the proportion of iNKT cell subsets that are activated (TABLE 2). The different tissue-homing preferences of the iNKT cell subsets therefore will have strong implications for how iNKT cell activation ultimately influences local immune responses.

Liver iNKT cells

Tissue residence and patrolling by liver iNKT cells.

Comparing all tissues in mice, iNKT cells are most frequent in the liver. NKT1 cells account for up to 40% of all intrahepatic lymphocytes, while the other iNKT cell subsets constitute a small fraction of the total lymphocyte count[35,69–71]. In the human liver, iNKT cells are also enriched but much less abundant than in mice; they have been reported to account for as much as 3–5% of intrahepatic T cells[72,73]. Several factors are important for the trafficking and long-term maintenance of liver iNKT cells, including IL-7 (REF.[74]), IL-15, the transcription factors homologue of BLIMP1 in T cell (HOBIT; also known as ZNF683), β-interferon gene positive regulatory domain I-binding factor (BLIMP1; also known as PRDM1), inhibitor of DNA binding 2 (ID2) and the cellular adhesion molecules intercellular adhesion molecule 1 (ICAM1) and lymphocyte function-associated antigen 1 (LFA1)[35,75–79]. The chemokine receptor CXC-chemokine receptor 6 (CXCR6) is important for survival and maintenance of iNKT cells in the liver[35,75–79]. CD69 is important for tissue residence of a variety of T cell populations, and CD69, along with HOBIT and BLIMP1, has been shown to be increased on non-circulating iNKT cells in the liver. HOBIT and BLIMP1 cooperate to maintain tissue residence by downregulating genes involved in tissue egress[80]. ID2 was shown to increase expression of CXCR6 by hepatic iNKT cells[79]. Blockade of LFA1 and ICAM1 reduced accumulation of iNKT cells in the liver and allowed for recirculation of liver iNKT cells in parabiotic mice[35].

At steady state, intravital microscopy studies have shown that iNKT cells patrol the liver sinusoids through a random, crawling motion both with and against the direction of blood flow[76] (FIG. 2a). Activation of the TCR, exposure to inflammatory cytokines such as IL-12 and IL-18 or tissue infection can trigger the arrest of patrolling iNKT cells on sinusoids[66,76,81] (FIG. 2b). CD1d is highly expressed on sinusoid-lining endothelial cells and, to a lesser extent, on Kupffer cells, the macrophages of the liver and tissue-resident and circulating DCs[76]. CD1d-mediated antigen presentation by several cell types can lead to activation and arrest of patrolling iNKT cells. Hepatocytes and stellate cells also express CD1d. These cells can present antigens to iNKT cells that enter the tissue, or they may interact with sinusoidal iNKT cells through openings in the endothelium[41,82] (FIG. 2). Notably, very recent data showed that hepatocyte-specific CD1d expression regulated hepatic iNKT cell homeostasis[41]. This function was dependent on the microsomal triglyceride transfer protein (MTP), which is involved in transfer of lipids onto CD1d in the endoplasmic reticulum. Absence of MTP or a hepatocyte-specific loss of CD1d was associated with a liver-specific increase in iNKT cell numbers, reduced iNKT cell apoptosis and a higher susceptibility to iNKT cell-mediated hepatitis[41].

Hepatic iNKT cells protect from infections.

Because of their prevalence in the liver, there are many examples in which iNKT cell activity plays an important role in local immune and inflammatory responses (TABLE 1). iNKT cell activation can be protective, as long as it is not chronic or excessive, as over activation leads to destructive tissue damage. One particularly well-studied example of beneficial liver iNKT cell activation is the response to *Borrelia burgdorferi* infection (FIG. 2c). *B. burgdorferi* is a

spirochaete that is the causative agent of Lyme disease. It expresses a well-characterized glycolipid antigen for iNKT cells[7,81,83]. After infection, patrolling liver iNKT cells slow their sinusoidal patrolling, with 80% of the iNKT cells becoming completely stationary within 5 hours[81]. At 8 hours, these arrested iNKT cells were found in intimate clusters with Kupffer cells in the liver sinusoids, which was dependent on surface expression of CXCR3 by iNKT cells, as well as Kupffer cell expression of CD1d and release of the CXCR3 ligand CXC-chemokine ligand 9 (CXCL9)[81]. At this time, CD69 was upregulated and IFNγ was produced by the iNKT cells[7,81]. Interestingly, while α-galactosyl ceramide (αGalCer) injection also induced arrest of iNKT cells and production of IFNγ and IL-4, it did not induce clustering with Kupffer cells[81]. This suggests that CD1d on other cell types also induces iNKT cell arrest in the vasculature, and it illustrates the important differences revealed when comparing the response to a pathogen versus a synthetic iNKT cell antigen[76,81].

When iNKT cell-deficient mice were infected with *B. burgdorferi*, there was a higher accumulation of spirochaetes in the joints, bladder, heart and liver, suggesting that iNKT cells help limit emigration of the spirochaetes out of the vasculature[81,84,85]. Joint inflammation was more pronounced in iNKT cell-deficient BALB/c mice, while carditis was more evident in iNKT cell-deficient C57Bl/6 mice[84–86]. IFNγ production by iNKT cells was increased after infection, and it probably plays a role in host protection in both the liver and the heart[84,85].

Similar protective effects have been shown in various hepatitis virus models. NKT1 cells inhibited hepatitis C virus (HCV) replication in the mouse liver, which also occurred through IFNγ production[87]. Humans with chronic HCV infection showed significantly reduced numbers of iNKT cells in the liver and peripheral blood compared with healthy controls in some studies[88,89], but this was not found in others[90]. The low frequencies of circulating iNKT cells in patients with chronic hepatitis B increased to normal levels when they achieved viral control[91]. Furthermore, in mouse models of expression of hepatitis B virus (HBV) antigens, HBV infection increased the production of autologous ligands that activate iNKT cells, and the absence of these cells led to reduced viral control[92]. Altogether, these data suggest a positive role for iNKT cells in protection against liver pathogens.

Hepatic iNKT cells play protective and contributory roles during inflammation.

In the case of chronic infections or chronic inflammation, even sterile inflammation, iNKT cell activity can be the cause of serious tissue damage. Injection of αGalCer-activated iNKT cells induced lethal inflammation in mice through the iNKT cell-mediated release of IFNγ, which stimulated the recruitment and activation of T_H1 cells and $CD8^+$ cytotoxic T lymphocytes. This T_H1 cell-type immune bias was also mediated by interactions between CD40 ligand (CD40L), which is increased on iNKT cells upon activation, and CD40 on DCs[72]. This interaction led to IL-12 production by DCs, which enhanced the cytotoxic effects of iNKT cells against hepatocytes[72]. Activated iNKT cells also caused hepatocyte death directly through increased FAS antigen ligand (FASL) expression as well as by the release of tumour necrosis factor (TNF), perforins and granzyme B[72,93] (FIG. 2d). Interestingly, following concanavalin A (Con A) injection, increased FASL also led to

apoptosis of iNKT cells[93], a process that was dependent on CC-chemokine receptor 5 (CCR5) expression by iNKT cells and suppression of their IL-4 production[94].

In models of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH), activation of the immune system contributed to progression of fatty liver damage. Because iNKT cells respond to lipid antigens and can exhibit cytotoxicity against hepatocytes, they have become a focus of research in this area. NASH-like liver pathology can be induced by choline-deficient, methionine choline-deficient or high-fat high-cholesterol diets, which led in each case to increased expression of CD1d, accumulation of intrahepatic iNKT cells and, as a result, increased fibrinogenesis, higher alanine aminotransferase (ALT) levels and higher NASH disease scores[73,95–97] (FIG. 2d). Notably, iNKT cell-deficient mice had dramatically less fibrosis and lower disease scores[95]. In humans, a similar correlation between increased iNKT cells, increased cirrhosis and higher NAFLD disease activity has also been shown[73,98]. Hepatic fibrosis during NASH has been shown to be dependent on IL-13, and some evidence suggests that iNKT cell-derived IL-13 contributes to this[73,99,100].

Similarly, using other hepatitis models, including chronic infection with hepatitis B virus and Con A-induced hepatitis, iNKT cells promoted liver fibrosis and prevented liver regeneration[93,101–103]. In these models, iNKT cells accumulated in the liver, upregulated CD69 and increased IL-12, IFNγ and TNF production[93,101–105]. Pathogenesis was ameliorated when iNKT cells were blocked or removed. This was shown by depletion of $NK1.1^+$ iNKT cells using an anti-NK1.1 antibody, by blockade of CD1d or in *Traj18*$^{-/-}$ mice, which lack the Jα (*Traj*) segment required to form the invariant TCRα. These data are consistent with the hypothesis that iNKT cells promote liver damage and actively block liver regeneration in this model[93,102,103]. iNKT cell recruitment and liver damage were also decreased in Con A-injected peroxisome proliferator-activated receptor-α (PPARα)-deficient mice, suggesting a role for PPARα in iNKT cell-mediated liver damage[105].

Comparable results were observed in a mouse model of primary biliary cirrhosis (PBC), where diseased mice had more iNKT cells, with increased CD69 expression and IFNγ production, which exacerbated liver injury[106,107]. These findings correlated with human data, which showed significantly higher numbers of circulating and liver-resident iNKT cells in patients with PBC[108].

During sterile hepatic injury caused by ischaemia reperfusion, there was an influx of iNKT cells, which contributed to tissue damage through IFNγ production[109]. This damage was ameliorated in iNKT cell-deficient mice and was restored by transfer of iNKT cells into *Rag1*$^{-/-}$ mice[109]. A different sterile liver injury model is caused by carbon tetrachloride injection leading to liver necrosis. In contrast to T_H1 cell-mediated damage, damage in this model was mediated by increased serum IL-17 and TNF, as well as by an influx of $IL\text{-}17^+$ iNKT cells[110]. These damaging immune responses were dampened by co-transfer of mesenchymal stem cells (MSCs), which induced an increase in serum IL-10 levels[110]. A similar effect was seen in an iNKT cell antigen-dependent acute liver injury model caused by αGalCer injection. Coadministration of αGalCer and MSCs reduced the hepatotoxicity of liver iNKT cells by suppressing $IL\text{-}17^+$ iNKT cells and inducing $IL\text{-}10^+$ and forkhead box

protein 3 (FOXP3)$^+$ regulatory iNKT cells in a paracrine indoleamine 2,3-dioxygenase (IDO)-dependent manner[110]. These data demonstrate that iNKT cell responses can be regulated through interactions with different cell types and their soluble factors. In LPS-induced hepatitis, liver iNKT cells induced a functional switch of myeloid-derived suppressor cells (MDSCs) from anti-inflammatory to pro-inflammatory function, thereby contributing to immune-mediated damage[111]. The results from some studies indicate that liver regeneration following partial hepatectomy is promoted by iNKT cells[112], or at least that it is CD1d-dependent[113]. Therefore, while having the potential for causing inflammatory damage, intrahepatic iNKT cells might also be intimately connected with homeostasis and regeneration of this tissue.

Notably, one study has suggested that iNKT cells also play a protective role in sterile injury healing, particularly in focal hepatic sterile thermal injury, in which healing was significantly delayed at 7 days after injury[114]. At 4 hours, iNKT cells approached the injury but made a startling 180° turn and retreated from the area[114]. At 8 hours, iNKT cells arrested and accumulated at the boundary of the injury site in a CD1d-dependent manner[114]. iNKT cells were retained there at 24 hours, which was dependent on IL-12 and IL-18, and they began producing IL-4. However, iNKT cells did not enter into the injured tissue until 48 hours[114]. The iNKT cell responses correlated with a switch from inflammatory monocytes to reparative monocytes around the lesion, which was dependent on IL-4 produced by iNKT cells[114], altogether suggesting a T_H2 cell-biased protective role for iNKT cells in this injury repair model.

iNKT cells in the lungs

Localization in blood vessels and lung tissue.

In mouse lungs, iNKT cells account for approximately 5% of resident lymphocytes, equating to 5×10^3 cells in C57Bl/6 mice. They establish resident, non-circulating populations within both the lung vasculature and the interstitial tissue[115]. The majority of the NKT1 and NKT2 cells were found in the vasculature, and by contrast, NKT17 cells were preferentially found within the smaller, interstitial tissue compartment[45,115,116] (FIG. 3). Overall, there is a proportionally higher frequency of lung iNKT cells in the tissue than other lymphocytes in the lung[116].

Factors required for iNKT cell homing to and maintenance within pulmonary tissue have yet to be determined. However, there is extensive evidence of the rapid extravasation of iNKT cells from the blood and into the tissue under a variety of activation conditions. Intranasal administration of αGalCer can induce iNKT cell extravasation owing to the upregulation of various chemokines, including CC-chemokine ligand 17 (CCL17), CXCL9 and CXCL13 (REFS[35,115]). Pulmonary infection with *Francisella tularensis* or *Streptococcus pneumoniae* has also been shown to induce iNKT cell expansion and vascular extravasation in a CCL2-dependent manner, suggesting that iNKT cells are recruited to and play a role within the lung interstitial tissue during infection[116–118].

Protection from pulmonary infections.

Similarly to infections in the liver, in several pulmonary bacterial infection models, including infections with *S. pneumoniae*, *Listeria monocytogenes* and *Pseudomonas aeruginosa*, iNKT cells were critical for protection in a CD1d-dependent manner (FIG. 3; TABLE 1). In the lungs, CD1d is expressed by several cell types, including alveolar macrophages, $CD11b^+$ DCs, $CD103^+$ DCs and monocyte-derived DCs, all of which are potential antigen-presenting cells for iNKT cells. During *S. pneumoniae* infection, iNKT cells increased in number and produced IFNγ and IL-17 within 13 hours of infection[8]. During *Schistosoma japonicum* parasitic infection, iNKT cell numbers surprisingly decreased; however, the remaining cells were more activated, as measured by both an increase in surface CD69 and CD25 and the secretion of cytokines, including IL-17, IL-10 and IL-5 (REF.[119]). Control of *Aspergillus fumigatus* in the lung was dependent on iNKT cells. The protective response required CD1d and IL-12, which were induced by antigen-presenting cell responses to the fungal wall β-glucan[120]. In these infection models and several others, in the absence of iNKT cells, pathogen burden was increased and survival decreased[8,65,118,121,122]. Furthermore, inflammatory responses were generally reduced following infection in iNKT cell-deficient mice, including decreased neutrophil recruitment and decreased synthesis of pro-inflammatory CXCL2 and TNF[118,121,122]. These data suggest that iNKT cells play a role in protective inflammatory responses to bacteria and parasites in the lungs.

iNKT cells also undergo activation and population expansion and show protective effects in pulmonary viral infections, such as those from influenza A virus. After infection with influenza A virus, mice lacking iNKT cells ($Cd1d^{-/-}$ mice or $Traj18^{-/-}$ mice) had increased viral loads and reduced survival compared with wild-type controls[123–125]. This correlated with a decrease in virus-specific $CD8^+$ T cells and antibodies. Survival, viral loads, $CD8^+$ T cell counts and antibody responses were recovered after injection of iNKT cells into $Traj18^{-/-}$ mice, but not after injection into $Cd1d^{-/-}$ mice, suggesting that the role of iNKT cells in protection is CD1d-dependent[123].

Despite the protective functions of iNKT cells in these infections, viruses have not been shown to contain or induce the synthesis of lipid antigens that stimulate iNKT cells via their TCRs[123–126]. There are several possible roles iNKT cells could play during viral infection. Upon activation, iNKT cells produce IFNγ, which can induce T_H1 cell-biased responses; this pathway is associated with the activation of NK cells, macrophages and $CD8^+$ T cells, all of which can aid in eliminating viruses[125]. The absence of iNKT cells also correlated with an increase in MDSCs in the lungs[123]. Adoptive transfer of iNKT cells reduced MDSC population expansion after influenza infection in a CD1d-dependent and CD40-dependent manner, suggesting that iNKT cells inhibit the immune-suppressive effects of MDSCs[123]. Additionally, iNKT cells produced IL-22 during infection, an IL-10 family cytokine that is protective against epithelial damage[63,127]. IL-22 and iNKT cells also played a role in protection from secondary *S. pneumoniae* infection following influenza, consistent with a protective role for iNKT cell-derived IL-22 (REFS[126,127]).

iNKT cells in asthma and allergies.

Activation of iNKT cells has been shown to exacerbate inflammation in the lungs in the absence of infection, as in the case of allergic asthma. Intranasal αGalCer administration led to increased numbers of iNKT cells, increased secretion of IFNγ and IL-4 by iNKT cells and acute lung inflammation[128]. When αGalCer was coadministered intranasally with ovalbumin (OVA), OVA challenge led to massive eosinophilia and an asthma-like response dominated by T_H2 cell-type cytokines[115]. In the OVA-induced model of allergic asthma with methacholine challenge, iNKT cells produced IL-4, IL-5, IL-10, IL-13 and IFNγ in an inducible T cell costimulator (ICOS)–ICOS ligand (ICOSL)-dependent manner[129,130]. In this model, *Traj18*$^{-/-}$ iNKT cell-deficient mice showed decreases in airway hyper-responsiveness, eosinophilia, IL-4, IL-5 and IL-13 in bronchiolar lavage fluid (BALF) and reduced anti-OVA IgE[130,131]. The decreases were reversed by adoptive transfer of iNKT cells[131]. NKT17 cells were a prevalent subset within the lung tissue, and upon stimulation with αGalCer, the IL-17 they secreted recruited neutrophils into the BALF but was not required for airway hyperresponsiveness[132,133].

As αGalCer is a highly potent antigen, the iNKT cell phenotypes seen in mouse models of asthma following exposure to αGalCer might not reflect what happens after the physiological stimulation of iNKT cells. In the absence of αGalCer, however, the induction of airway eosinophilia and mucus in response to ragweed was reduced in CD1d-deficient mice[134], and airway hyper-reactivity induced by sensitization with OVA and alum was also dependent on CD1d and iNKT cells[131,135]. In addition, ozone-induced asthma was similarly dependent on iNKT cells and T_H2 cell cytokines but also required IL-17 (REF.[136]). Furthermore, sterile house dust extracts (HDEs) contain antigens for iNKT cells[137], and alum adjuvant could be replaced by HDEs to sensitize for OVA-induced airway hyper reactivity. This adjuvant-like activity of HDEs was dependent on iNKT cells[137].

iNKT cells have also been shown to play roles in driving chronic obstructive pulmonary disease (COPD). Repeated administration of αGalCer resulted in a decline of lung function and COPD-like symptoms, such as mucus hyper-secretion, fibrosis and emphysema[128]. Patients with COPD were found to have increased numbers of iNKT cells in the blood, and mice chronically exposed to cigarette smoke had increased numbers of iNKT cells in the lung[138]. These data indicate that oxidative stress may contribute to iNKT cell activation. Moreover, in a model of post-viral asthma and COPD induced by infection with Sendai virus, iNKT cells contributed to disease by inducing IL-13 production by macrophages[139].

However, despite these reports cited above, there is not universal agreement on the importance of iNKT cells for asthma models in mice. One study investigated several different models of airway disease and found no significant difference between wild-type and iNKT cell-deficient mice in any of the models tested[140]. A potential explanation for these discrepancies is differences in the micro-biota, in the lung or elsewhere in the body, in different mouse colonies. Germ-free mice showed increased iNKT cells in the lung, which was attributed to increased CXCL16 expression[141]. These mice also had more severe OVA-induced asthma than specific-pathogen free (SPF) mice, suggesting a correlation between the micro-biota, iNKT cells and asthma[141]. Whether the frequency of iNKT cells is increased in the lung tissue and BALF of patients with asthma has been a controversial

issue[142,143], and it remains to be determined whether iNKT cells are relevant for the pathogenesis of human asthma.

Adipose tissue and iNKT cell function

Distinct iNKT cells are found in adipose tissue.

The concept that T cells that recognize self-lipids might have a special role in regulating adipose tissue is appealing, but there is no consensus on the function of iNKT cells in fat tissue. In both humans and mice, iNKT cells were found to be enriched in visceral adipose tissue (VAT), where they represented up to 15–20% of total T cells[56,144–146]. The data on human iNKT cells is particularly striking, because they tend to be much less frequent in other sites in humans and generally less frequent than they are in mice. iNKT cells were also enriched in mouse subcutaneous, perirenal and epididymal adipose tissue[147]; at this latter site, iNKT cells were shown to express higher amounts of CD69 and IFNγ than splenic iNKT cells[148]. Similarly to iNKT cells in other sites, adipose iNKT cells do not recirculate[34]. Unlike in the liver, however, they do not depend on LFA1 or ICAM1 expression for their retention, and the factors required for their recruitment and retention in adipose tissue remain unknown[34].

In adipose tissues, CD1d is highly expressed on M2 macrophages and to a much lesser extent on M1 macrophages, DCs and eosinophils[149]. Adipocytes express a high amount of mRNA encoding CD1d, and they can present glycolipid antigens in vitro to iNKT cells[146]. The iNKT cells in adipose tissue are characterized by an increased frequency of IL-10-producing cells[34,56]; one report also described adipose tissue-associated iNKT cells as having low expression of PLZF and high expression of the transcription factor E4BP4, which induces *Il10* transcription[34]. IL-10-producing iNKT cells, which are also referred to as NKT10 cells, could be induced by repeated or strong antigenic stimulation[56,150]. Mice with a mutation in the constant region of TCRβ that influences TCRα–TCRβ pairing had a higher percentage of iNKT cells in adipose tissue, although the percentage of iNKT cells was lower in other sites, such as spleen[57].

Adipose iNKT cells and metabolic regulation.

There are few data regarding the response to infection by iNKT cells in fat; instead, the available evidence indicates a role for iNKT cells in the homeostasis of adipose tissue. In obese humans, iNKT cells were reduced in the circulation compared with in lean, healthy, age-matched controls[144,145]. Fewer adipose tissue iNKT cells in humans also correlated with an increased body mass index (BMI) and glucose intolerance[151]. Similarly, iNKT cells were decreased in the fat of obese mice[144,146,151]. iNKT cell numbers recovered in the adipose tissue of humans after bariatric surgery, and in obese mice, they increased after the animals were removed from a long-term high-fat diet (HFD)[144,146,151]. Taken together, these findings suggest a positive correlation between obesity and decreased iNKT cell numbers[144], and several mechanisms have since been suggested.

One such mechanism suggests a role for iNKT cells in the ‘browning’ of white adipose tissue (WAT). Activation of iNKT cells with αGalCer led to significant weight loss in obese

mice[147]. This correlated with an increase in thermogenesis, an overall 1 °C rise in body temperature and a significant induction of mitochondrial brown fat uncoupling protein 1 $(UCP1)^+$ cells in inguinal WAT within 24 hours of injection[147]. This browning was partially dependent on iNKT cell-induced fibroblast growth factor 21 (FGF21), which had previously been shown to induce weight loss in humans and mice[147]. Notably, this iNKT cell-induced browning mechanism was not dependent on αGalCer, and could be induced by adoptive transfer of iNKT cells into obese mice[147]. Similarly, liraglutide, a glucagon-like peptide 1 receptor (GLP1R) agonist, also led to proliferation of adipose iNKT cells and iNKT cell-dependent FGF21 induction and weight loss[147], further suggesting that an iNKT cell-induced FGF21-mediated pathway leads to weight loss.

There are controversies regarding the regulation of the inflammatory state of adipose tissue by iNKT cells (TABLE 1). In some studies of mice on a low-fat diet, which is considered to be a normal diet, adipose tissue iNKT cells had an activated phenotype, which was modulated directly by adipocytes, and they secreted IL-4 and IL-13 (REF.[152]). *Cd1d*$^{-/-}$ mice on a normal diet had an insulin-resistant phenotype, without obvious adipose tissue inflammation, suggesting a protective role for iNKT cells in adipose tissue[152]. Similar protective effects of iNKT cells were seen following very short-term exposure of mice to a HFD, during which time iNKT cells increased in the adipose tissue and promoted M2 macrophage polarization[151,153]. It may seem paradoxical that iNKT cells had a similar effect under both conditions, but the HFD was maintained for only 4 days in the short-term study. *Cd1d*$^{-/-}$ mice showed impaired systemic glucose tolerance and insulin sensitivity after 4 days, 8 weeks or 24 weeks of a HFD[151]. Similar effects were seen in *Traj18*$^{-/-}$ mice. However, two studies that fed mice a HFD for a much longer time, 12–13 weeks, found a contradictory response, in which iNKT cells in the WAT and VAT drove a pro-inflammatory response, including secretion of IFNγ, IL-4 and TNF, which led to recruitment of M1 macrophages into the adipose tissue[154,155]. This effect was exacerbated by treatment with αGalCer[154,155]. Consistent with this, *Traj18*$^{-/-}$, *Cd1d*$^{-/-}$ and *B2m*$^{-/-}$ mice had higher infiltration of M2 macrophages and less M1 macrophages and were better protected from glucose intolerance, insulin resistance and excessive lipid accumulation[154,155]. Taken together, these studies suggest that iNKT cells promote tissue inflammation and insulin resistance rather than protect against their development. However, in another study, where *Cd1d*$^{-/-}$ mice were fed a HFD for 26 weeks, there was no difference in glucose or insulin tolerance, and expression of genes encoding pro-inflammatory markers was similar to controls[156]. In agreement with this neutral outcome, another group found that *Traj18*$^{-/-}$ mice gained less weight than wild-type mice after 10 weeks on a HFD, but iNKT cells did not affect glucose clearance[157].

It is known that inflammation in fat plays a fundamental role in the development of insulin resistance[158,159], which is mediated by the balance of M1 and M2 macrophages in the tissue[160,161]. M2 macrophages were enriched in lean animals and had anti-inflammatory functions by promoting the secretion of IL-4, IL-10 and IL-13. Therefore, one mechanism whereby iNKT cells influence the state of adipose tissue is through their interactions with macrophages. In an investigation in which iNKT cells were beneficial, on either a standard diet or a HFD, *Traj18*$^{-/-}$ and *CD1d*$^{-/-}$ mice had higher levels of pro-inflammatory cytokines, higher infiltration of pro-inflammatory M1 macrophages in the fat and a reduction

in anti-inflammatory M2 macrophages[144]. Adoptive transfer of iNKT cells or treatment with αGalCer protected mice from weight gain, increased IL-10 production, reversed glucose intolerance and improved overall metabolic health[144]. Furthermore, in vivo stimulation of adipose iNKT cells with αGalCer led to expansion of iNKT cells and production of IL-10 and IL-4, which can induce an M2 phenotype on macrophages[34,144,151]. Interaction with M2 macrophages induced production of IL-4 and IL-13 by iNKT cells, while interactions with M1 macrophages induced iNKT cell pro-inflammatory IFNγ production[149]. Furthermore, with in vivo *Cd1d* deletion specifically on M2 macrophages, using a $Cd1d^{fl/fl}$ mouse crossed to a $Lyz2^{Cre}$ mouse strain, M2 polarization of macrophages was inhibited, and so-called meta-inflammation and glucose intolerance were promoted. *Cd1d* deletion on M1 and M2 macrophages had the opposite effect and promoted M2 macrophage polarization and inhibited meta-inflammation[149]. These data led to the hypothesis that iNKT cells could play a role in modulating macrophage polarization in the fat through interactions with CD1d expressed by these cells, which could have profound downstream implications for physiology.

There is also evidence that CD1d expressed by adipocytes plays a role in the regulation of metabolism, presumably by presenting autologous antigens that regulate the homeostasis of adipose tissue. Here again, however, the results from different reports are not consistent. Obesity and insulin resistance were aggravated in mice with an adipocyte-specific deletion of *Cd1d* after 8 weeks of HFD feeding[144,162], implying physiologically important antigen presentation by CD1d expressed by adipocytes in vivo. In another study, removal of CD1d expression from adipocytes reduced rather than increased obesity and insulin resistance[163].

There could be several reasons for the striking discrepancies in these results, including the genetics of the mouse strains, diet composition and the microbiome. One recent investigation has aimed to understand these opposing conclusions and found that the interactions between iNKT cells and macrophages depended on the length of time the mice were on a HFD. After 8 weeks of HFD, M2 macrophages in the visceral and subcutaneous adipose tissue had decreased expression of CD1d; however, a majority of iNKT cells were still colocalized with M2 macrophages[149]. This shifted by 16 weeks, when the M1:M2 macrophage ratio and levels of TNF and IL-1β increased, causing a shift towards iNKT cell interaction with M1 macrophages and leading to a pro-inflammatory environment[149]. These results suggest that iNKT cells play a protective role in the early stages of obesity, when they are interacting predominantly with M2 macrophages, and a pathogenic role at later stages of obesity, when they are interacting with M1 macrophages.

Intestinal iNKT cells

Intestinal iNKT cells shape the microbiota.

In the intestine, variable numbers of iNKT cells have been reported, owing in part to differences in how the cells were defined. Early studies defined iNKT cells as either $CD3^{+}NK1.1^{+}$ or $TCR\beta^{+}NK1.1^{+}$ and found that they were more prevalent in the intraepithelial lymphocyte (IEL) compartment than in the lamina propria[70,164,165]. With the advent of CD1d tetramers, however, a more accurate detection was possible, and it was found that iNKT cells in mice were more prevalent in the lamina propria than in the

epithelial layer and more highly represented in small intestine than in large intestine. Generally, iNKT cells accounted for less than 1% of the total lymphocytes in the small intestine[71,166,167]. In humans, iNKT cells are also mainly found within the lamina propria and estimated to represent less than 0.4% of small intestinal T cells[166]. In the human fetus, mature IFNγ-producing iNKT cells were found to be present in the small intestine by 18 weeks of gestation[168]. This suggests that iNKT cells differentiate and acquire effector functions even before establishment of the commensal microbiota.

While they arise before microbial colonization, it is clear that iNKT cells contribute to the regulation of intestinal tissue homeostasis through crosstalk with the intestinal microbiota, and the mechanisms involved have begun to be elucidated[169]. The presence of CD1d and iNKT cells in the intestine was found to alter the composition of the intestinal microbiota[42], and CD1d expression restrained the ability of bacteria to colonize the intestine[170] by influencing Paneth cell release of antimicrobial peptides. This may be due to iNKT cell secretion of IFNγ, as this cytokine triggers release of antimicrobial peptides[171]. CD1d-deficient mice were colonized at an accelerated rate with several bacteria, including *P. aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Lactobacillus gasseri*[172]. iNKT cell-deficient mice also showed a significant reduction of certain unclassified members of the order Bacteroidales and members of the family Sutterellaceae[42]. This was shown to be mediated by CD1d expression by a $CD11c^+$ cell type[42], which also played a role in physically segregating the microbiota from epithelial cells[42].

Influences of the microbiota on intestinal iNKT cells.

As well as shaping the microbiota, iNKT cells are themselves influenced by the microbiota. Germ-free mice were shown to have higher numbers of iNKT cells in both the intraepithelial and the lamina propria compartments owing to increased CXCL16 expression in the intestine[141]. The iNKT cells from germ-free mice were hypo-responsive compared with those from SPF mice[141,173]. Antibiotic-treated mice also had increased intestinal iNKT cells[174]. In the context of inflammatory disease, germ-free mice had increased morbidity in oxazolone-induced colitis[141], an inflammatory bowel disease (IBD) model that requires iNKT cell-derived IL-13 (REFS[141,175]) (TABLE 1). Humans with ulcerative colitis also showed higher IL-13 production by lamina propria CD1d-reactive T cells, but these cells did not express invariant TCRα[176]. Reintroduction of a conventional microbiota to neonatal mice, but not to adult mice, normalized iNKT cell populations and reversed the susceptibility to colitis[141]. The common commensal bacteria *Bacteroides fragilis* produces a glycosphingolipid antigen that is structurally similar to αGalCer[177]. Monocolonization of neonatal germ-free mice with *B. fragilis*, or even exposure to the purified glycolipid antigen from this bacterium, restored iNKT cell numbers in the intestine to normal levels and protected them from hypersensitivity to oxazolone-induced colitis[178]. It is uncertain whether this glycolipid antigen is an antagonist, is an agonist or has mixtures of these properties[177,178]; this requires further investigation with synthetic rather than purified material. In another study, however, introduction of *Sphingomonas* spp. bacteria, which contain characterized iNKT cell antigens, to adult germ-free mice restored the maturation and responsiveness of intestinal iNKT cells[173]. Furthermore, mice that underwent short-term antibiotic treatment, followed by re-colonization through faecal transplant with a dysbiotic

microbiota from mice with dextran sulfate sodium-induced colitis, had not only an increase in intestinal iNKT cells but also an increase in the percentage of these cells that produced IFNγ[174]. Therefore, alterations in the intestinal microbiota of adult mice can affect intestinal iNKT cells, although alterations in very young mice may have more widespread and longer lasting effects.

Intestinal inflammation and infection.

iNKT cells in the small intestine are also important in several other contexts. Coeliac disease is mediated by IFNγ production in response to gluten. In one study, patients with coeliac disease had increased numbers of iNKT cells in the duodenal IEL compartment, as measured by RNA expression of the invariant chain rearrangement (*TRAV10–TRAJ18*)[179]. This increase positively correlated with the severity of the mucosal lesions and mRNA for IFNγ[179]. However, a conflicting study found decreased numbers of iNKT cells in the duodenum of patients with coeliac disease, and these iNKT cells showed a lower cytokine-producing capacity[180]. Intestinal iNKT cells have been shown to have a pathological role in *Toxoplasma gondii* infection. Oral ingestion of *T. gondii* promotes a T_H1 cell-biased immune response in the gut, but excessive IFNγ production caused lethal, acute ileitis in C57Bl/6 mice[167]. iNKT cell-deficient *Traj18*$^{-/-}$ mice showed decreased levels of IFNγ in the intestine, decreased ileitis and improved survival, suggesting that iNKT cells contribute to tissue damage[167]. By contrast, iNKT cells seemed to show tissue-protective functions in the intestine of *Apc*$^{Min/+}$ mice, which spontaneously develop benign polyps, an early step in colorectal cancer carcinogenesis. *Apc*$^{Min/+}$ mice deficient in iNKT cells had increased intestinal polyps, with iNKT cells apparently preventing polyp formation by suppressing local inflammation[181]. The iNKT cells in the polyps produced IL-10 and shared some of the phenotypical features of iNKT cells in adipose tissue[34,55,56,144].

In summary, iNKT cells in the intestine can be either pro-inflammatory or anti-inflammatory, and the type of antigen-presenting cell engaging them via CD1d expression seems to be critical for determining their function. When CD1d was deleted from CD11c$^+$ cells, there was a decrease in the frequency of NKT17 cells in the small intestine lamina propria, while NKT1 cells remained unaltered[42], suggesting differential control of the homeostasis of iNKT cell subsets by different CD1d-expressing cell types. In another study, deletion of CD1d expression from intestinal epithelial cells contributed to increased inflammation, while deletion from bone marrow-derived cells had the opposite effect[182]. A perhaps similar phenomenon applies to the liver, where deletion of CD1d from hepatocytes leads to an increased number of intrahepatic iNKT cells and increased inflammation following Con A hepatitis[41]. Therefore, in influencing tissue homeostasis, iNKT cells interact with both haematopoietic and non-haematopoietic cell types, and CD1d expression by non-haematopoietic cells is anti-inflammatory in both the liver and the intestine.

Perspective and discussion

As described in detail above, in some contexts, iNKT cells promote host defence and inflammatory reactions in peripheral sites, whereas in other contexts, they act as regulatory cells that inhibit inflammation (TABLE 1). We are just beginning to understand the

mechanisms underlying this puzzling diversity of influences that iNKT cells have on the immune response. A major advance has been the finding that there are functional subsets of iNKT cells that are similar in their cytokine secretion both to T helper cell subsets and to subsets of ILCs.

Despite this progress, a number of important questions regarding the formation and stability of the iNKT cell subsets remain unanswered. First, while the transcriptomes of mature NKT1, NKT2 and NKT17 cell subsets that take up long-term residence in the thymus are known to be highly different, it remains to be determined how similar the transcriptional programmes of the peripheral iNKT cell counterparts in the circulation are. Additionally, how mature and terminally differentiated are the iNKT cells that recently emigrated from the thymus, and to what extent do they continue to differentiate in the periphery? Second, how stable are these subsets, and can they interconvert? Third, what iNKT cells are the precursors of those cells that seem to depend on peripheral antigenic stimulation, such as NKT_{FH} and NKT10 cells? Additionally, what are the factors that dictate homing and maintenance of iNKT cells in different tissue sites?

While the definition of the functional subsets of iNKT cells has provided a foundation for understanding their diverse function in different tissues, the type of activating stimulus is equally important. For example, an NKT1 cell stimulated by cytokines, such as IL-12 and IL-18, will produce IFNγ, but TCR activation of the same cell type induces a panoply of cytokines, including IL-4 and IFNγ. Therefore, understanding the role of iNKT cells in homeostasis and pathogenesis in different tissues requires not only an accounting of subset frequencies but also a systematic analysis of the local environment, including whether the iNKT cell is in blood or parenchymal tissue, the types of antigen-presenting cells and cytokines that are present and importantly the exposure of the tissue environment to microbial components, which will be very different in adipose tissue compared with liver or intestine. A deeper knowledge of iNKT cell subset formation and stability, combined with insight into the aspects of the tissue milieu, will help in understanding the immune modulatory capacity of not only iNKT cells but also other populations of innate-like T cells, such as mucosal-associated invariant T cells and $\gamma\delta$ T cells, and undoubtedly will help efforts to use these populations in a therapeutic context (BOX 1).

Acknowledgements

The authors' work is supported by US National Institutes of Health (NIH) grants AI 71922, AI 105215 and AI 137230. C.M.C. is suqpported by American Lung Association Senior Research Training Fellowship RT-412662. The authors thank their colleagues for many helpful discussions.

Reviewer information

Nature Reviews Immunology thanks P. Brennan, M. Brenner, A. Lehuen and the other anonymous reviewer(s) for their contribution to the peer review of this work.

Glossary

α-Galactosyl ceramide
(αGalCer). A glycosphingolipid that is a specific and highly potent activator of invariant natural killer T cells.

Concanavalin A
(Con A). A mitogenic lectin that stimulates T cell proliferation and activation and has been shown to induce invariant natural killer T cell-activated liver damage.

Myeloid-derived suppressor cells
(MDSCs). A heterogeneous group of myeloid cells that exhibits strong immunosuppressive function.

Visceral adipose tissue
(VAT). Adipose tissue that is located around internal organs in the abdominal cavity. Excess visceral fat has been linked to insulin resistance and other obesity-related diseases.

White adipose tissue
(WAT). White and brown adipose tissues are the two types of adipose tissue found in mammals. WAT is the primary tissue for energy storage and it also serves roles in whole body thermal insulation and endocrine regulation of energy homeostasis.

Meta-inflammation
A metabolically driven, chronic, low-grade inflammation that is manifested by immune cells and adipocytes. This inflammation has been linked to obesity and insulin resistance.

Mucosal-associated invariant T cells
An innate-like T cell type with an invariant T cell receptor α (TCRα) chain that recognizes vitamin B metabolites presented on the MHC class I-like molecule MHC class I-related gene protein (MR1).

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Box 1 |

iNKT cells are a promising target for immune therapies

Several features of invariant natural killer T (iNKT) cells make them a promising and versatile target for immune therapy. First is their ‘one-size fits all’ specificity, meaning an effective glycolipid antigen for iNKT cells will work in everyone. Second, because CD1d is not polymorphic[13,183,184], transferred iNKT cells will not cause graft-versus-host disease. Additionally, iNKT cells have strong effector function, they are tissue homing, they are self-renewing in the periphery, and they can be grown rapidly in vitro. Much of the clinical and biotech effort has been on boosting the immune response to cancer[15–17,185–188], although rapid iNKT cell effector responses are also being tested to achieve adjuvant-like stimulation of vaccine responses[189–192]. Inhibition of iNKT cells may prove to be valuable for preventing inflammation in the liver or for crisis in sickle cell disease[193,194]. These strategies are focused on the NKT1 cell-like functions of human iNKT cells, such as IFNγ production and cytotoxicity. Because their immune responses vary, iNKT cell activation is also being tested as a means to prevent graft-versus-host disease through an antigen formulation that stimulates IL-4 and other cytokines[195,196]. The main obstacles are that iNKT cells are very infrequent in some individuals, and therefore are difficult to expand, that human responses to α-galactosyl ceramide (αGalCer) are not as robust as in mice and that the types of cytokines produced by iNKT cells vary[188,197]. These problems may be obviated by current efforts to synthesize more potent antigens or ones that stimulate a more a T helper 1 (T_H1) cell-type polarized iNKT cell response[186,191,198]. Alternatively, iNKT cells can be expanded in vivo by using specific anti-T cell receptor (TCR) antibodies, and ex vivo-expanded iNKT cells are being tested as a cancer treatment[199,200]. iNKT cells may also be a useful platform for expression of chimeric antigen receptors (CARs); for example, a CAR that is specific for neuroblastomas would target these cells, and at the same time, the natural CD1d autoreactivity of the iNKT cells enables them to kill suppressive tumour associated macrophages[15–18]. Time will tell whether any of these diverse strategies will provide an effective immune therapy.

Box 2 |

Follicular helper NKT cells provide cognate B cell help

Follicular helper natural killer T (NKT_{FH}) cells are essentially absent in mice that have not been immunized. However, 6 days after α-galactosyl ceramide (αGalCer) immunization, NKT_{FH} cell populations were detected in the spleen and lymph nodes and expanded in a CD1d-dependent manner[53,201]. Furthermore, the NKT_{FH} cells established prolonged contact with B cells[53]. These cells show similar phenotypes and localization patterns to follicular helper T (T_{FH}) cells, with shared features including expression of CD4, CXC-chemokine receptor 5 (CXCR5) and programmed cell death 1 (PD1), and they could be found in germinal centres following immunization with αGalCer[53]. Also similar to T_{FH} cells, the development of NKT_{FH} cells is dependent on expression of the transcription factor B cell lymphoma 6 (BCL-6), CD28-mediated co-stimulation and the presence of B cells[53]. NKT_{FH} cells were also found in human tonsil, where approximately 10% of the iNKT cells had high co-expression of PD1 and CXCR5 (REF. [53]). Immunization with αGalCer linked to the hapten nitrophenyl led to antigen-specific germinal centre formation by 3 days, and NKT_{FH} cells produced IL-21 by day 5 (REF. [53]). This is a faster rate than conventional T cells, which typically take 10 days after protein antigen immunization, and is more similar to kinetics of T cell-independent germinal centres[53]. There is limited evidence of invariant natural killer T (iNKT) cells driving long-term IgG responses. While cognate NKT_{FH} cells drove plasmablast and germinal centre formation, affinity maturation and a robust primary IgG antibody response dependent on iNKT cell-derived IL-21, NKT_{FH} cells could not generate long-lived plasma cells or memory B cells[53,54]. Injection of mice with liposomes containing either *Streptococcus pneumoniae* capsular polysaccharide or αGalCer activated long-lasting IgG1 responses and memory responses upon antigen recall[202]. However, these responses were largely extrafollicular, and there was minimal NKT_{FH} cell differentiation[202]. It is notable that iNKT cells also provide non-cognate B cell help, as shown by the role of iNKT cell-derived IL-4 in promoting germinal centre formation during influenza infection[47].

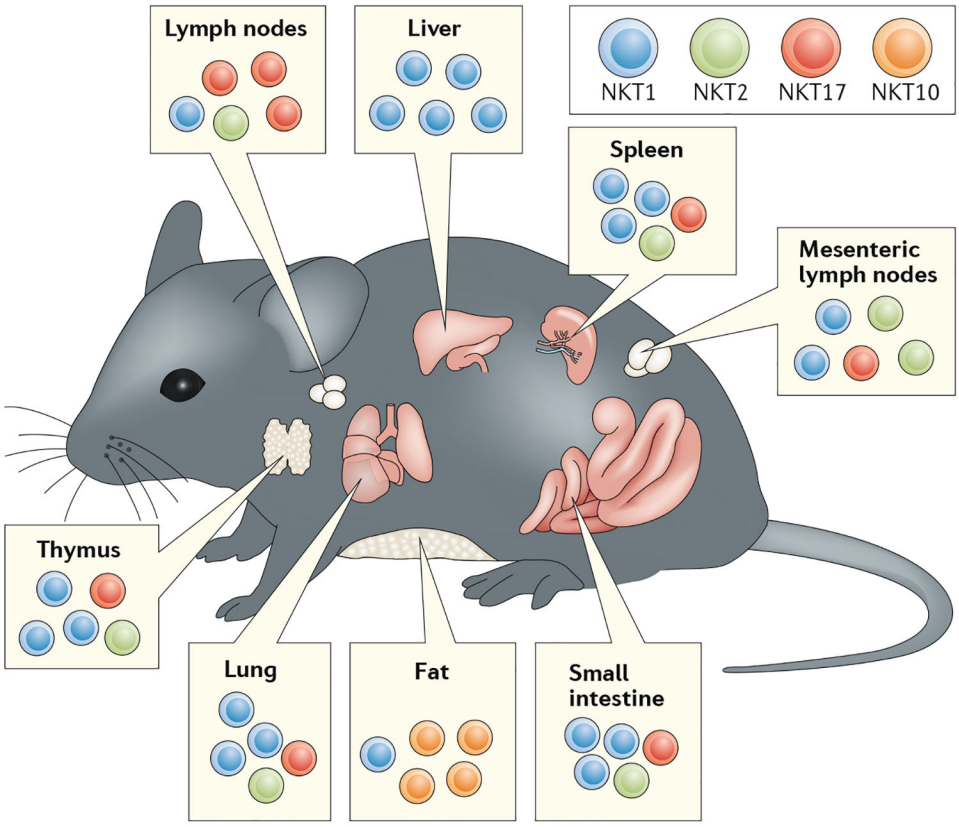

Fig. 1 |. Tissue distribution of iNKT cell subsets in mice.

The figure illustrates how distinct invariant natural killer T (iNKT) cell subsets preferentially localize in lymphatic and non-lymphatic tissues in C57Bl/6 mice. NKT1, NKT2, NKT17 and NKT10 cells are indicated in blue, green, red and orange, respectively. The figure depicts the relative frequency of each iNKT cell subset in different tissue sites, including liver, lungs, intestine and adipose tissue, which are the focus of this Review.

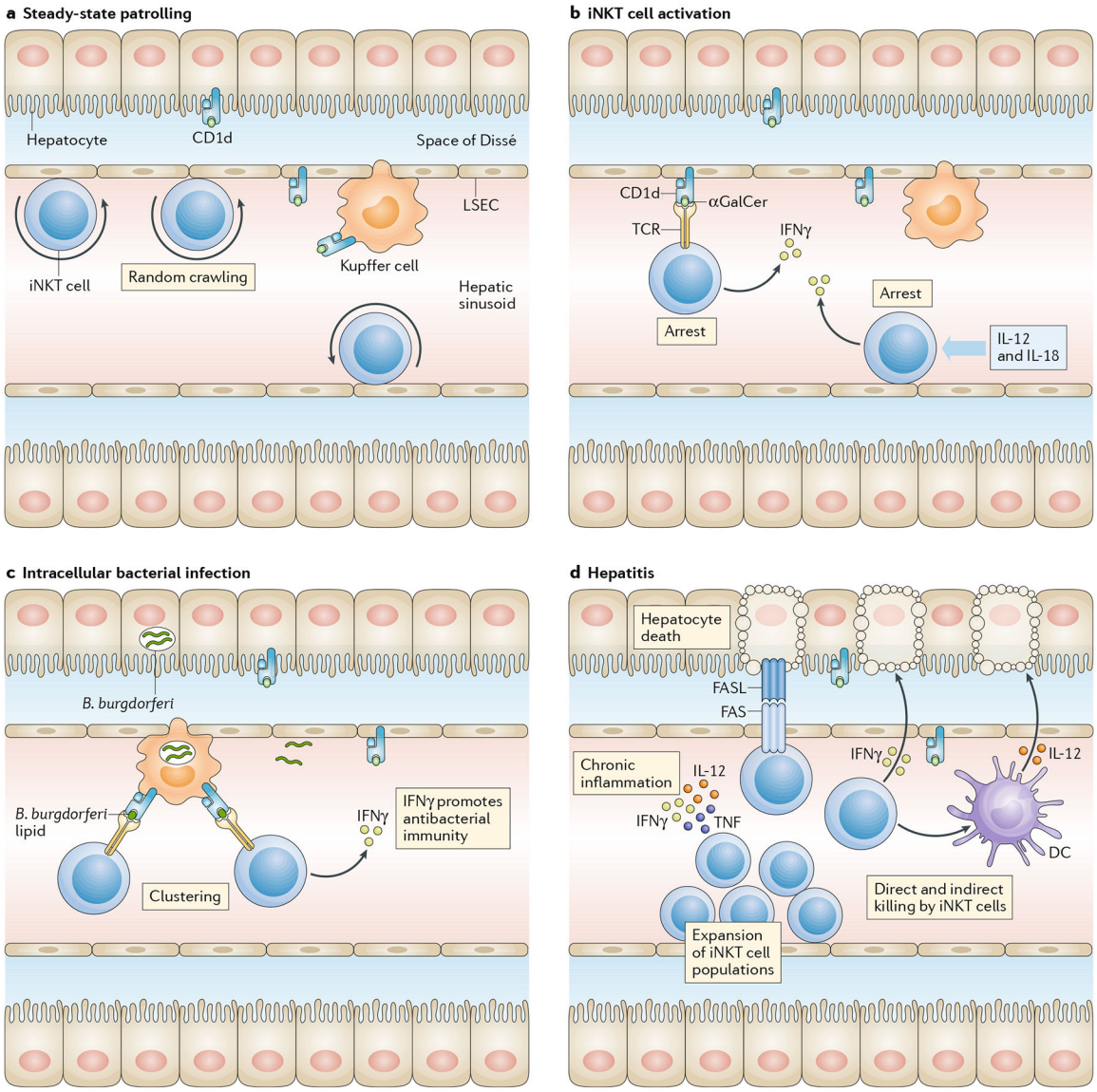

Fig. 2 |. iNKT cells in the liver sinusoids.

a | At steady state, invariant natural killer T (iNKT) cells patrol the liver sinusoids in a random crawling motion both with and against the flow of blood. CD1d is expressed on liver sinusoidal endothelial cells (LSECs), Kupffer cells, hepatocytes and other cells not indicated in the figure. **b** | Activation of iNKT cells by α-galactosyl ceramide (αGalCer) or cytokines (such as IL-12 and IL-18) causes arrest of the cells on LSECs and production of IFNγ. **c** | During *Borrelia burgdorferi* infection, the bacterial spirochaete is taken up by Kupffer cells and can enter the liver parenchyma. *B. burgdorferi* infection causes the arrest and expansion of iNKT cell populations, as well as their production of IFNγ. The activated iNKT cells form clusters with Kupffer cells in a CD1d-dependent manner. **d** | Chronic hepatic inflammation results in the expansion of iNKT cell populations and the production of IFNγ, IL-12 and tumour necrosis factor (TNF) by various cell types in the liver. iNKT cells can induce death of hepatocytes directly by apoptosis-mediating surface antigen FAS–FAS antigen ligand (FASL) interactions, or indirectly through production of IFNγ. IFNγ

production by iNKT cells can also stimulate dendritic cells (DCs) to produce IL-12, which can lead to killing of hepatocytes. TCR, T cell receptor.

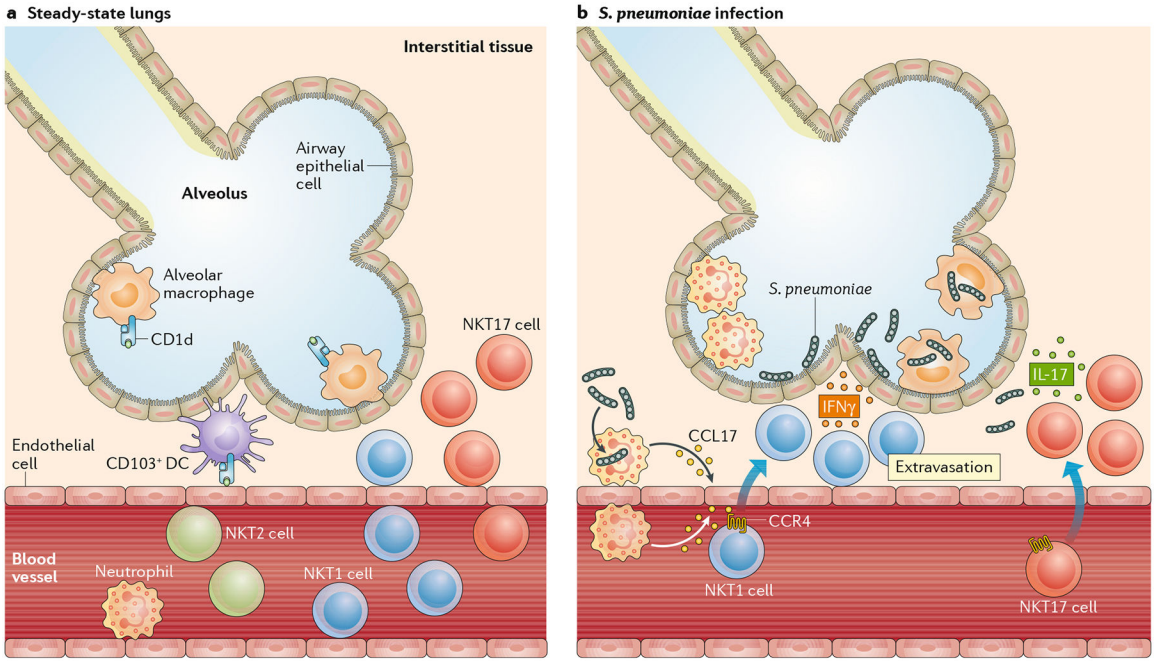

Fig. 3 |. iNKT cells in the lung.

a | In the steady-state lung of C57Bl/6 mice, NKT1 cells are the most numerous invariant natural killer T (iNKT) cell subset, and they are localized primarily in the vasculature; the same is true for the less abundant NKT2 cell subset. NKT17 cells are mainly found within the lung tissue, although there is also a small number that is found in the vasculature. In the airways, CD1d is expressed by alveolar macrophages, $CD11b^+$ dendritic cells (DCs), $CD103^+$ DCs and monocyte-derived DCs. **b** | Following pulmonary infection with *Streptococcus pneumoniae*, NKT1 and NKT17 cells rapidly extravasate into the lung tissue, and these populations expand. These processes are driven by chemokines, including CC-chemokine ligand (CCL17) from neutrophils, which draws iNKT cells into the tissue. Within 13 hours, NKT1 cells produce IFNγ and NKT17 cells produce IL-17. Additional neutrophils are recruited into the tissue and alveoli, and this recruitment is decreased when iNKT cells are absent. These pathways promote clearance of *S. pneumoniae* from the lungs. CCR4, CC-chemokine receptor 4.

Table 1 |

Responses and roles of iNKT cells in different tissues during infection or disease

Tissue	Disease or infection model	iNKT cell response	Effect of iNKT cell response	Refs
Liver	*Borrelia burgdorferi* infection	• Expansion of iNKT cells • IFNγ production • Induction of Kupffer cell clustering	Protective	7,81,83–86
	Hepatitis C virus infection	• Expansion of iNKT cells • IFNγ production	Protective	87–89
	Nonalchoholic steatohepatitis and fibrosis	• Increased CD1d expression • Accumulation of hepatic iNKT cells • IL-13 production	Pathological	73,95–98
	Hepatitis	• Increased CD1d expression • Accumulation of hepatic iNKT cells • IL-13 production	Pathological	93,101–105
	Sterile hepatic injury	• IFNγ production • IL-17A and TNF production	Pathological	109,110
Lungs	Pulmonary infection with *Streptococcus pneumoniae, Listeria monocytogenes* or *Pseudomonas aeruginosa*	• iNKT cell expansion • CD1d-dependent neutrophil recruitment • CXCL2 and TNF production	Protective	8,117,121,122,126,203
	Influenza virus	• iNKT cell population expansion • CD1d-dependent production of IFNγ and IL-22	Protective	63,123–127
	Asthma	• IFNγ and IL-4 production • IL-17A production • ICOS-dependent production of IL-4, IL-5, IL-10 and IL-13	Pathological (debated)	115,128–135,138–143
Adipose tissue	Obesity	• IL-4 and IL-13 secretion • NKT cells decreased in patients who are obese	Protective	144–146,151

Nat Rev Immunol. Author manuscript; available in PMC 2019 January 23.

Tissue	Disease or infection model	iNKT cell response	Effect of iNKT cell response	Refs
	Insulin resistance	Increased iNKT cell numbers led to increased IL-4 and IL-13 secretion and M2 macrophage polarization	Protective	144,151–153,162
	Insulin resistance	• Increased IFNγ and TNF production • Increased M1 macrophage polarization	Pathological	154,155
Intestine	Oxazolone-induced colitis	IL-13 production by iNKT cells	Pathological	178
	Colorectal cancer polyps	IL-10 production by iNKT cells to reduce inflammation	Protective	181

CXCL2, CXC-chemokine ligand 2; ICOS, inducible T cell costimulator; iNKT, invariant natural killer T; TNF, tumour necrosis factor.

Table 2 |

Key characteristics of the main iNKT cell subsets

iNKT cell subset	Transcription factors	Cell surface markers	Cytokines secreted	Refs
NKT1 cells	• PLZF^{low} • T-bet^{+}	• CD49a^{+} • CD122^{+} • CXCR3^{+} • CD4 ($^{+/-}$)	• IFNγ • IL-4 • TNF	19–21,24
NKT2 cells	PLZF^{hi}	• ICOS^{+} • CD4^{+}	• IL-4 • IL-5 • IL-13	19–21,24
NKT17 cells	• PLZF^{int} • RORγt^{+}	• ICOS^{+} • Syndecan 1^{+} • CD4^{-} • CCR6^{+} • CD103^{+} • NRP1^{+}	• IL-17 • IL-22 • GM-CSF • TNF	19–21,24,204
NKT$_{FH}$ cells	BCL-6^{+}	• CXCR5^{+} • PD1^{hi} • CD4^{+}	IL-21	53
NKT10 cells	• PLZF^{-} • E4BP4^{+}	• CD49d^{+} • PD1^{+} • NRP1^{+}	• IL-10 • IL-2	34,55,56

BCL-6, B cell lymphoma 6 protein; CCR6, CC-chemokine receptor 6; CXCR, CXC-chemokine receptor; E4BP4, E4 promoter-binding protein 4; GM-CSF, granulocyte–macrophage colony-stimulating factor; ICOS, inducible T cell costimulator; iNKT, invariant natural killer T; NKT$_{FH}$, follicular helper natural killer T; NRP1, neuropilin 1; PD1, programmed cell death 1; PLZF, promyelocytic leukaemia factor; TNF, tumour necrosis factor.